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THE IDENTIFICATION AND DIFFERENTIATION OF SOME OF THE MUSCLE RELAXANTS

by

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "The Identification and Differentiation of Some of the Muscle Relaxants," submitted by Lynn A. E. Doan in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

A series of specific physical criteria, by which eleven of the muscle relaxants currently in popular clinical application can be identified and differentiated is presented.

Fifty five derivatives of these compounds were prepared using a variety of characterizing reagents which included phenyl and 1-naphthyl isocyanate, 3,5-dinitrobenzoyl chloride, 3-nitrophthalic anhydride, 2,4-dinitrobenzenesulfenyl chloride, xanthydrol, benzhydrol, acetic anhydride, picric acid,ammonium reineckate, sodium tetraphenylborate, chloroplatinic acid, and methyl iodide.

Melting point data of these derivatives was supplemented with the infrared spectra in an effort to provide additional, alternative paramaters for characterization purposes.

Microchemical tests were undertaken for five muscle relaxants containing the amine moiety, and results were presented pictorially as photomicrographs. Such common alkaloidal and basic nitrogenous reagents as picric, styphnic, picrolonic, and chloroplatinic acids, ammonium reineckate, and potassium iodide were employed.

ACKNOWLEDGMENTS

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION AND LITERATURE SURVEY

HISTORICAL

The search for drugs capable of diminishing skeletal muscle tone and involuntary movement has led to the introduction during recent years of a variety of agents capable of a relatively weak, centrally mediated muscle relaxation. The object was to obtain drugs which abolish spasticity, tremor, and similar excessive motor activity without interfering with normal tone and movement.

A large number of compounds act centrally to decrease excessive motor activity; these include general anaesthetics, barbituates, apomorphine and its congeners, benzazole and its derivatives, the belladonna alkaloids, mephenesin and its congeners, and the synthetic antiparkinsonism drugs (1). But only the last two categories have a sufficiently selective action on the central nervous system to permit their being classed as centrally acting skeletal muscle relaxants.

1) Glycols and Derivatives as Muscle Relaxants

Gilbert and Descomps (2) and Launoy (3) in 1910 observed that phenoxypropanediol, known in France as "Antodyne", caused reversible flaccid paralysis in animals. However, almost four decades elapsed before interest in this property of the glycerol ethers was renewed as a result of the studies of Berger and Bradley (4,5) in 1947. They investigated the pharmacological properties of a large number of simple mono ethers of glycerol and found that certain of these compounds possessed a peculiar central depressant action. These workers suggested that the 1-o-tolyl ether of glycerol (mephenesin), the most suitable and safest compound of the series may be useful in the treatment of spastic and hyperkinetic states and for the production of muscle relaxation during anesthesia.

The greatest disadvantage of mephenesin was its short duration of action. After it was established that the evanescent action of the drug was due to the oxidation of mephenesin in the body to the physiologically inactive 2-(o-toloxy) lactic acid (6), various related compounds were prepared in which blockage of the hydroxyl groups by various radicals made this method of inactivation inoperative (7). Transformation of the hydroxyl group to a carbamate moiety yielded compounds of some interest. Thus the carbamate of 3-o-toloxy-1,2-propanediol and 1-o-toloxy-2-propanol exhibited a stronger paralyzing activity than mephenesin but unexpectedly, did not possess a longer duration of action (8). During the same period it was noted that certain 2,2-disubstituted-1,3-propanediols, while possessing pharmacological properties similar to those of mephenesin, had in addition, a particularly intense action at supraspinal levels (9,10).

In 1950, Berger and Ludwig (11) described the anticonvulsant action of 2,2-diethyl-1,3-propanediol (DEP), and some of its homologues and esters. The dicarbamate derivatives of these 2,2-disubstituted-1,3-propanediols were long lasting, although most of the substances had only a low order of activity (12). Among these, 2-methyl-2-n-propyl-1,3-propanediol dicarbamate, to which the generic name "meprobamate" was assigned, was unusual in possessing both pronounced muscle relaxant and potent anticonvulsant properties and in exerting a marked taming effect on monkeys (8,13,14,15).

Ginzel *et al.* (16), compared the pharmacological activity of 3-(o-methoxyphenoxy)-1,2-propanediol (guaicol glyceryl ether, GCE) with mephensin and pointed out that its potency as a spinal cord depressant and anticonvulsant is comparable with mephensin but that it has greater water solubility and a much lower hemolytic activity. GCE however, has a duration of activity about equal to mephensin, probably because of a similar metabolic alteration. Impressed with

the advantages of safety and therapeutic convenience of this compound, Truitt and Little (17), decided to study the pharmacological effects of the carbamate ester of GCE, namely, 3-(o-methoxyphenoxy)-1,2-propanediol monocarbamate, later to be called generically, methocarbamol. Their studies showed methocarbamol to have a significantly longer duration of action than mephenesin and in addition, it exceeded both mephenesin and mephenesin carbamate in its protection against electro-shock convulsions.

A number of phenyl alkyl carbamates with various substituents on the phenyl ring and the nitrogen atom as well as the second carbon were prepared during the period 1954 to 1959 (18). Among these compounds, 2-hydroxy-2-phenyl ethyl carbamate (styramate) possessed interesting pharmacological effects on the central nervous system and was investigated by De Salva *et al.* (18,19).

The replacement of a hydrogen atom on one of the carbamyl nitrogens of meprobamate with an isopropyl group by Berger and Ludwig *et al.* (20,21), yielded N-isopropyl-2-methyl-2-propyl-1,3-propanediol dicarbamate, called carisoprodal.

2) Aminoalcohols as Muscle Relaxants

A reduction of voluntary muscle spasm through a central depressant effect has been shown by some compounds which act peripherally as cholinergic blocking agents. These drugs are used primarily in the relief of rigidity and spasticity of paralysis agitans (Parkinson's disease). Examples of this group of drugs include biperiden, cycrimine, procyclidine and trihexyphenidyl.

Paralysis agitans or parkinsonism, first described by the English physician James Parkinson in 1817, is a condition that is currently treated with anticholinergic drugs. The symptomatic relief afforded by drugs of the belladonna group in parkinsonism has been recognized

since the days of Charcot in 1874 (22). Until recently the most widely used preparations were atropine, hyoscyamus and strammonium, which must be pushed to the limit of tolerance to obtain the optimum results. In high doses they produced unpleasant side-effects, such as dryness of the mouth, paralysis of visual accommodation, drowsiness, dizziness, and constipation. These reasons prompted a vigorous search for potentially useful agents in various series of synthetic spasmolytic compounds. With the introduction of the newer spasmolytic drugs it was hoped that these would be as effective as the belladonna preparations and without their side effects.

One of the initial and most successful drugs has been trihexyphenidyl, which was introduced in 1949. This compound was synthesized by Denton and his co-workers (23), and its pharmacological properties were first disclosed during the study of a series of synthetic antispasmodic agents by Cunningham _et al._ in 1949 (24). The initial clinical trials as a drug in the treatment of Parkinson's disease were reported by Corbin (25) and Doshay and Constable (26). The introduction of trihexyphenidyl was an important advance in therapy but its effectiveness was limited.

Cycrimine hydrochloride was introduced in 1953, and was synthesized according to the procedure of Denton _et al._ (27). Magee and De Jung (28) were among the first to carry out clinical trials, and describe a series of patients with paralysis agitans, who were treated with this drug.

Procyclidine hydrochloride is a spasmolytic compound chemically related to trihexyphenidyl and originally prepared by the method of Adamson (29). It has been employed with success in the treatment of Parkinson's syndrome (30).

Biperiden hydrochloride has been used for a number of years in Germany in the treatment of Parkinson's disease and related disorders (31). The pharmacology of the compound is described by Haas and Klavehn (32).

3) Miscellaneous

Several compounds of unique structure have been found to possess skeletal muscle relaxant properties through some central action. O'Dell *et al.* (33) during a pharmacological investigation of a new series of 2-substituted pyridine derivatives (34,35), found that some of the series possessed analgesic activity as well as considerable muscle relaxant action. Certain selected derivatives, especially 2-(β-hydroxyphenethylamino)-pyridine (phenyramidol) was found to be the most promising of the series. It may be considered as a nitrogen isostere of the 1,2-glycol derivatives (e.g., styramate). This compound produces muscle relaxation through the same pharmacological mechanism as mephenesin and its congeners. Since this compound is related chemically and pharmacologically, to the glycols, it will be discussed in later parts of this thesis as though being part of the glycol grouping.

CHEMISTRY and PHARMACOLOGY

The compounds of interest to this thesis fall into two distinct chemical groupings which differ not only in chemical composition but also in their method of inducing muscle relaxation. However, they have in common the ability to diminish skeletal muscle tone and involuntary movement by an action on the central nervous system.

1) Glycols and Derivatives

Mephenesin is the prototype drug of the centrally acting skeletal muscle relaxants of the glycol grouping. The pharmacological actions of the compound are common to all members of the group. It has limited therapeutic usefulness because of its transient action and untoward effects when administered intravenously, but it has been an important tool, and has stimulated the search for similarly acting compounds with improved properties. The more recently introduced drugs of this class have a longer duration of action than mephenesin, which is partly the result of a slower rate of absorption from the gastrointestinal tract. However, some of these drugs are also less readily detoxified and thus stay in the plasma and tissues for longer periods (36).

These drugs relax skeletal muscles through a rather specific action on the central nervous system, and therefore, the muscle relaxation produced is brought about in a completely different manner than that produced by curare and its analogues which act at the myoneural junction. These centrally acting compounds block impulses at the interneurons of polysynaptic reflex arcs, mainly at the level of the spinal cord. Thus, they markedly reduce multisynaptic but not monosynaptic, spinal reflexes (37). Neurons of the brain stem, thalamus, and basal ganglia may also be depressed.

When administered to experimental animals, these drugs can cause

decreased motor activity, ataxia, loss of the righting reflex, flaccid paralysis, and in toxic doses, death due to respiratory failure. The margin of safety between the toxic and therapeutic doses is relatively wide.

Experimental evidence seems to indicate that mephenesin and its congeners raise synaptic resistance in certain polysynaptic pathways (36). Therefore, it should be theoretically possible to administer doses of these drugs that would lessen excessive reflex excitability in certain disorders without significantly reducing the flow of tonic motor impulses to skeletal muscles. In fact, it has been demonstrated that spasticity, tremor and excessive motor activity can be abolished without interfering with normal tone and movement.

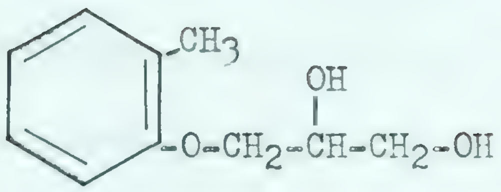
Muscle relaxants of this group have been advocated in the management of almost every musculoskeletal and neuromuscular condition which is characterized by painful muscle spasm as exemplified by bursitis, spondylitis, disk syndromes, sprains, strains, and low back pain.

There is little real evidence to indicate that these drugs have any place in the long range treatment of Parkinson's disease or any similar condition marked by neuronal damage.

Certain members of this group have been used as adjuncts to psycho-therapy in the treatment of anxiety tension states. Meprobamate is the ideal example of this.

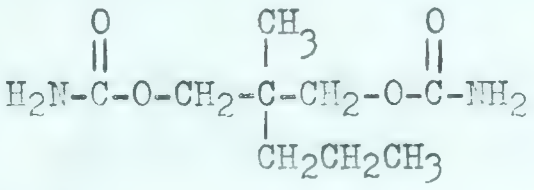
Compounds of the glycol type utilized in this problem will be described briefly in monographs containing their generic name, trade name (in parentheses), and structual formula.

(a) Mephenesin B.P.C. (38), N.F. (39), N.N.D. (36); (Tolserol), 3-<u>o</u>-toloxy-1,2-propanediol.



Mephenesin was prepared from 3-chloro-1,2-propanediol and sodium <u>o</u>-cresolate by the Wheeler and Wilson (40) synthesis for 1-phenyl ethers of glycerol. Mephenesin is a white, odorless powder with a bitter taste. The drug is sparingly soluble in water but freely soluble in alcohol and most organic solvents. It produces muscle relaxation and has mild sedative properties, both of short duration.

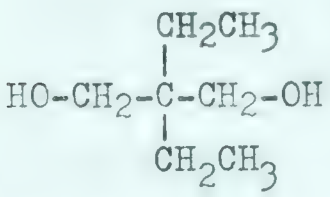
(b) Meprobamate U.S.P. (41), B.P. (42), N.N.D.; (Equanil, Miltown), 2-methyl-2-propyl-1,3-propanediol dicarbamate.



$$H_2N-\overset{O}{\overset{\|}{C}}-O-CH_2-\underset{CH_2CH_2CH_3}{\overset{CH_3}{C}}-CH_2-O-\overset{O}{\overset{\|}{C}}-NH_2$$

Meprobamate was produced initially by Ludwig and Piech (43). It is a white, essentially odorless, bitter powder, relatively insoluble in water and freely soluble in alcohol and most organic solvents. The drug is stable in the presence of dilute acid or alkali. The duration of muscle relaxation effect produced by meprobamate is eight to ten times longer than that produced by mephenesin, but of the same type.

(c) DEP; (Prenderol), 2,2-diethyl-1,3-propanediol.

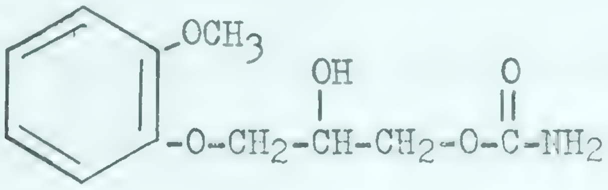


$$HO-CH_2-\underset{CH_2CH_3}{\overset{CH_2CH_3}{C}}-CH_2-OH$$

McKusick (44) and Shortridge <u>et al</u>. (45) independently prepared DEP in the same year. DEP is a white crystalline, odorless solid not appreciably soluble in water but is very soluble in ethyl ether, alcohol or hot benzene. Solutions of the drug are stable and can be sterilized. Its effects in certain neuromuscular disorders are similiar to mephenesin, also the

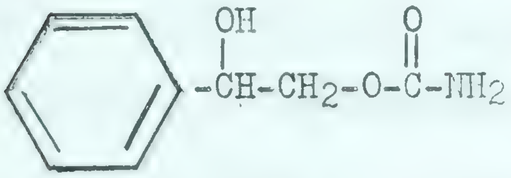
duration of action is relatively short due to rapid inactivation.

(d) Methocarbamol N.N.D.; (Robaxin), 2-hydroxy-3-o-methoxyphenoxypropyl carbamate.



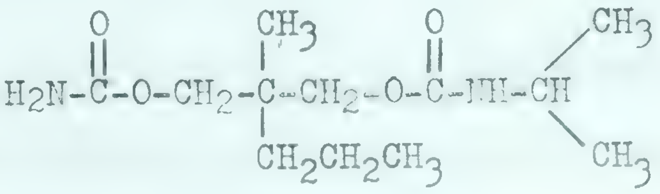
Methocarbamol was prepared by Murphey (46) and is a white crystalline, odorless solid. It is not appreciably soluble in water, but is soluble in alcohol and propylene glycol. Peak plasma concentrations of methocarbamol are reached more slowly (one hour) than for mephenesin (thirty minutes) but are more sustained.

(e) Styramate N.N.D.; (Sinaxar), 2-hydroxy-2-phenylethyl carbamate.



Bossinger et al. (18) were responsible for the synthesis of this compound. It is the carbamate of an ethylene glycol and is related in structure to mephenesin carbamate. After oral administration the duration of action of styramate seems to be two or three times that of mephenesin.

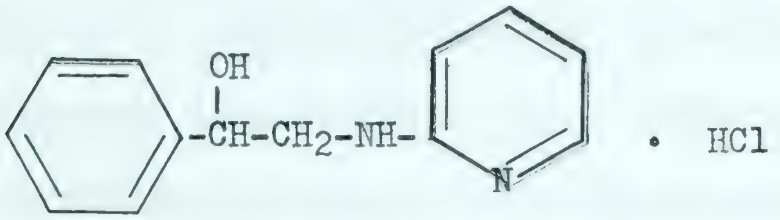
(f) Carisoprodal N.N.D.; (Rela, Soma), N-isopropyl-2-methyl-2-propyl-1,3-propanediol dicarbamate.



Berger and Ludwig et al. (20,21) were the first to prepare this compound and test its pharmacological activity. Carisoprodal is a bitter, odorless, white crystalline solid which is relatively insoluble in water and freely soluble in most organic solvents. On boiling with strong acid or alkali, carisoprodal undergoes hydrolysis, but it is stable in dilute acid or alkali.

In man, its onset of action usually occurs in thirty minutes after administration, and effects last as long as six hours. The compound is a centrally acting skeletal muscle relaxant, and in addition possesses analgesic action in certain types of pain.

(g) Phenyramidol Hydrochloride N.N.D.; (Analexin), 2-(β-hydroxyphenethylamino)-pyridine hydrochloride.



Gray and Heitmeier (34,35) during a study of a new series of 2-substituted pyridine derivatives synthesized phenyramidol. It is a white, crystalline almost odorless solid which is very soluble in water and alcohol. The compound may be considered a nitrogen isostere of the 1,2-glycol derivatives (e.g., styramate). Phenyramidol possesses good analgesic properties as well as muscle relaxant properties.

2) Aminoalcohols

The development of aminoalcohols as parasympatholytics has taken place during the past fifteen years, with most of the research being directed toward finding useful parkinsonlytics. All of the useful compounds have had the general characteristic of possessing rather bulky groups around the hydroxyl function, together with a cyclic amino function. Another structural feature common to all aminoalcohol anticholinergics studied, is the α-aminopropanol arrangement with three carbons intervening between the hydroxyl and amino functions. All of the aminoalcohols employed in the treatment of paralysis agitans are tertiary amines and, because the desired locus of action is central, quaternization of the nitrogen destroys the antiparkinsonism properties. However, quaternization of these aminoalcohols has been utilized to enhance the anticholinergic activity and to

produce antispasmodic and antisecretory compounds. The marked difference in activity by simple quaternization is shown vividly by the comparison of procyclidine with its methylchloride, tricyclamol chloride. The former is a useful drug in parkinsonism, but the latter has very little value.

The aminoalcohols produce no inhibition of transmission through neuromuscular pathways (no blockage of polysynaptic reflexes) and therefore the muscle relaxation produced is unlike that induced by the agents discussed earlier (37). These synthetic drugs are primarily a therapeutic tool in the relief of rigidity and spasticity of paralysis agitans (Parkinson's disease).

It is an old empirical observation that belladonna alkaloids have beneficial effects in the syndrome known as parkinsonism, which consists of muscular rigidity and tremor. Parkinsonism often follows encephalitis, cerebral atherosclerosis, or poisoning with carbon monoxide or manganese. A lack of complete understanding of the exact mechanism of this syndrome has made it difficult to develop drugs for its management on a purely rational basis. In general, the pharmacological approach to the treatment of this disease consists of investigating various atropine-like drugs in the hope that, if they are not superior in effectiveness to the belladonna alkaloids, they may at least have fewer side effects.

Although the mode of action of the atropine-like drugs as antitremor agents is not definitely known, it obviously reflects a central mechanism attack. The activity is confined to those compounds that can pass the blood-brain barrier (i.e., tertiary amines and not quaternary ammonium compounds). There are some postulations to the effect that acetylcholine is a neurohumoral agent in the central nervous system as well as peripherally, and that anticholinergics can block its action in either locus (47).

Perhaps the most significant observation to be made in the clinical

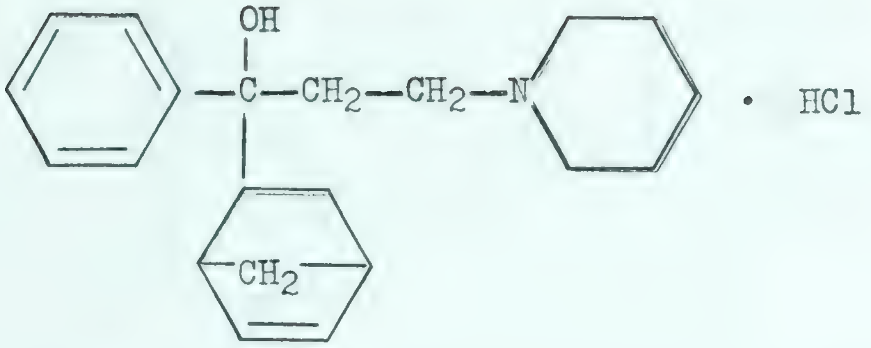
study of paralysis agitans is that each patient's care must be strictly individualized. One patient will derive considerable benefit from a particular drug, while another may find that same substance is entirely ineffective. In addition, the physician must consider the individual variation to drug tolerance. Consequently, the larger the number of preparations available, even though of limited range of therapeutic efficacy, the greater opportunity there is to provide a satisfactory program for each patient. By virtue of the aforementioned reasons, work was carried out and newer drugs were introduced.

Although many compounds have been introduced for treatment of the parkinson syndrome, there is apparently a dire need for additional agents which will provide a more potent action, less side effects, and a wide assortment of replacement for drugs, which seem to lose their efficacy with the passing of time.

The synthetic aminoalcohol antiparkinsonism drugs reduce muscular rigidity, steady the hands, prevent immobility of the eyeballs, improve the gait, and elevate the mood. Side reactions are similiar to those encountered for other anticholinergic drugs; dryness of the mouth, dizziness, blurring of vision, gastric irritation, and drowsiness.

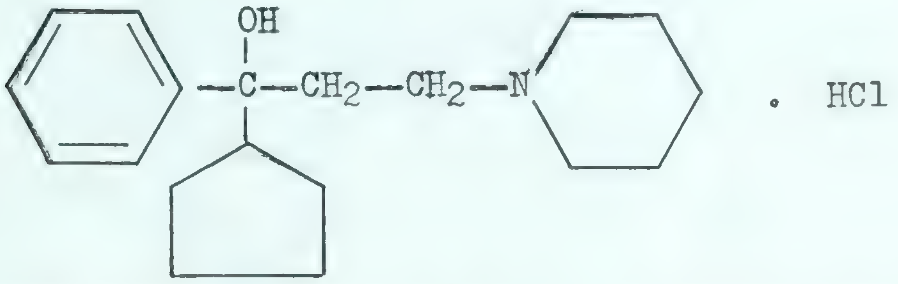
The tertiary aminoalcohols investigated in this problem will be described briefly in monographs containing their generic name, trade name (in parentheses) and molecular formula.

(a) Biperiden Hydrochloride N.N.D.; (Akineton Hydrochloride), $\underline{\alpha}$-bicyclo(2.2.1)-hept-5-en-2-yl-$\underline{\alpha}$-phenyl-1-piperidino-propanol hydrochloride.



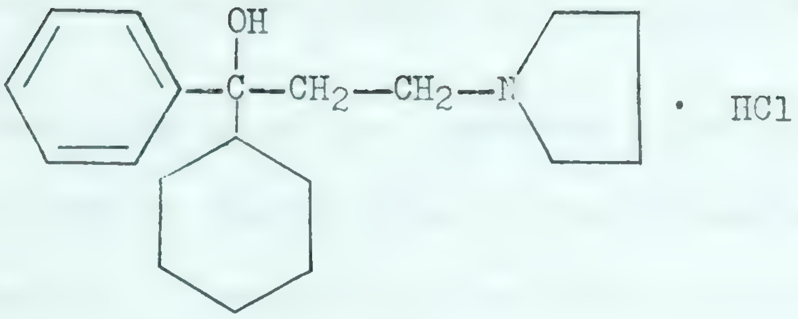
This compound is described by Haas and Klevehn (32) and is a white, crystalline, odorless powder which is practically insoluble in water and alcohol. Biperiden has a relatively weak visceral anticholinergic action but a strong nicotinolytic action in terms of its ability to block nicotine-induced convulsions. The drug is used in all types of Parkinson's disease.

(b) Cycrimine Hydrochloride N.F., N.N.D.; (Pagitane Hydrochloride), 1-cyclopentyl-1-phenyl-3-piperidino-1-propanol hydrochloride.



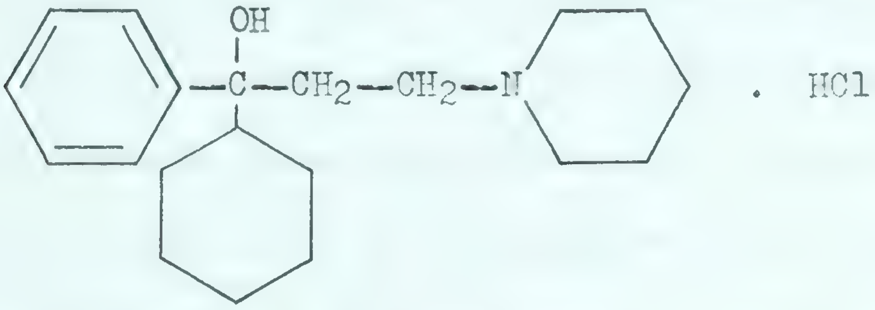
This drug was made according to the procedure of Denton _et al_. (27) and occurs as a white, odorless, bitter solid which is sparingly soluble in alcohol and only slightly soluble in water. A 0.5% solution in water is slightly acidic. Cycrimine has an antispasmodic activity of about one fourth to one half that of atropine sulphate and it is a useful addition to the class of drugs employed in the symptomatic treatment of paralysis agitans.

(c) Procyclidine Hydrochloride B.P., B.P.C., N.N.D.; (Kemadrin), 1-cyclohexyl-1-phenyl-3-pyrrolidino-1-propanol hydrochloride.



This compound was prepared according to the method of Adamson (29) and occurs as white crystals which are moderately soluble in water (3:100) but are more soluble in alcohol or chloroform. Procyclidine which has low toxicity is an effective peripheral anticholinergic although its chief clinical usefulness lies in the treatment of Parkinson's syndrome.

(d) Trihexyphenidyl Hydrochloride B.P., B.P.C., N.N.D., U.S.P.; (Artane Hydrochloride, Benzhexol Hydrochloride), 1-cyclohexyl-1-phenyl-3-piperidino-1-propanol hydrochloride.



This compound which was synthesized by Denton and his co-workers (23) occurs as a white, odorless crystalline compound that is soluble in water 1:100. However, it is more soluble in alcohol or chloroform. Introduced in 1949, it is approximately one half as active as atropine as an antispasmodic, but it has lesser side-effects. The drug has found a place in the treatment of parkinsonism and its most striking effect is the reduction of rigidity.

METHODS for QUALITATIVE and QUANTITATIVE DETERMINATION

The majority of the literature survey was concerned with the quantitative rather than the qualitative determination of the muscle relaxants in question. It was found that much work had been done concerning individual members of the group, while other compounds received little or no investigation. The survey failed to yield any identification schemes dealing with the qualitative differentiation of the muscle relaxants as a group.

No official compendium recognizes all eleven of the skeletal muscle relaxants studied in this work. The identification tests that are described or referred to in the official compendia, are generally provided only as an aid to identification, and are not sufficient to establish proof of identity. Such tests as anion (chloride) precipitation reactions are of no value. More specific physical and chemical reactions make use of such criteria as melting points (i.e., parent compounds, free base, or the acid salt), and in one instance the melting point of an easily prepared derivative. On this basis, it would seem therefore that there are no comprehensive official criteria for the qualitative determination of these compounds.

For convenience, the literature survey will be discussed under the generic name of each compound.

1) Mephenesin

Rajeswaran and Kirk (48) have attempted to develop a comprehensive identification scheme for various tranquilizing and related drugs. They have reported ultraviolet spectra, color reactions, and photomicrographs of some fifty tranquilizers which included mephenesin. The wavelength of maximum ultraviolet absorption has been used by Bradford and Brackett (49) as a means of identification for various dangerous drugs and poisons

and similarly, Udenfriend et al. (50) subjected a large number of drugs to spectrophotofluorometric study as a means of identification. Fluorometric and spectrophotometric methods have also been developed for the quantitative estimation of mephenesin (51-53). Titrimetric analysis of phenyl glycerol ethers is described by Stross and Stuckey (54) and can be applied to mephenesin. A volumetric quantitative assay of mephenesin is outlined by Waaler and Paulssen (55) using periodic acid.

2) DEP (Prenderol)

A few isolated derivatives have been reported for prenderol (56-58) but none of these were developed in an organized or correlated identification scheme and thus were probably not meant to be used for qualitative analysis directly.

3) Meprobamate

References dealing with the application of chromatography comprise the majority of the literature dealing with the qualitative determination of meprobamate. Paper chromatographic methods utilizing a variety of conditions and developing reagents are described in the literature (59-64). In addition, thin layer chromatography has been a useful and rapid method of identifying meprobamate (65-67). Kazyak and Knoblock (68) have applied gas chromatography to analytic toxicology and presented gas chromatographic data on several compounds including meprobamate.

Cooper (69) and Maggiorelli (70) have outlined relatively non specific color reaction which may assist in the identification of this agent.

Derivatives have been used to some degree for the indentification of this compound as indicated by the recommendations of the B.P., B.P.C., and U.S.P. that the diacetate be made. Workman (71) suggested the formation of the diacetate derivative of meprobamate, together with the infrared spectra of the parent compound as a means of indentification.

Several workers (72-74) have charaterized meprobamate by the preparation of the dixanthyl derivative and Penprase and Biles (75), utilized photomicrographs to identify various sedatives and anticonvulsants including meprobamate.

Spectrophotometric techniques appear to be the most valuable for the quantitative estimation of meprobamate. Several references (76-80) describe a colorimetric method based on the formation of a colored complex between furfural or p-dimethylaminobenzaldehyde in the presence of antimony trichloride or sulfuric acid.

Agranoff et al. (81) measured the urinary excretion of meprobamate by the formation of a colored complex, while Harris and Reik (82) also developed a qualitative procedure applicable to urine. The latter authors hydrolyzed meprobamate in alkaline solution, and after acidification the ammonia produced is determined by nesslerization. Ellis and Hetzel (83) also described a colorimetric micro-method for urine while other authors (84,85) have employed infrared spectrophotometry to estimate meprobamate. The B.P., and U.S.P. describe procedures based on a reaction that hydrolyses the meprobamate molecule into two fragments, one of which can be determined by formal titration.

4) Methocarbamol

Although this compound has been studied from pharmacological and clinical points of view, very little information is available for its identification or estimation.

Pico and Bastus (86) discuss the infrared spectrum of methocarbamol and also outline an ultraviolet spectrophotometric method for the simultaneous determination of phenobarbital and methocarbamol. A colorimetric technique is described by Devaux et al. (87), and can be applied to methocarbamol and other nonsubstituted nitrogen carbamates. Morgan et al.

(88) estimated methocarbamol by hydrolysis, with subsequent periodate oxidation to formaldehyde, and colorimetric determination with chromotropic acid. Parikh and Mukherji (89) found that methocarbamol forms bromoderivatives and exploited this property, under controlled conditions, to develop a suitable quantitative method for its estimation.

5) Styramate

No methods of qualitative or quantitative estimation of this compound could be found in the literature.

6) Carisoprodal

Douglas and Schlosser (60) published paper chromatrographic studies of both carisopradal and meprobamate, while in another investigation Douglas *et al.* (90), examined the metabolic fate of carisoprodal by carrying out infrared studies and paper chromatograms as a means of indentification. A colorimetric method for the determination of carisoprodal in biological fluids was introduced by Kato *et al.* (91).

7) Phenyramidol Hydrochloride

No methods of qualitative or quantitative estimation of this compound could be found in the literature. A few isolated derivatives have been reported for phenyramidol (34,92,93), and these can be used as an aid to its estimation.

8) Biperiden Hydrochloride

No methods of qualitative or quantitative estimation of this compound could be found in the literature.

9) Trihexyphenidyl, Cycrimine, and Procyclidine Hydrochloride Salts

Since these three compounds have been combined in most of the recent investigations concerning their qualitative differentiation, they will be considered as one group.

Clark (94) presented a comprehensive microchemical indentification scheme for some forty-eight atropine like drugs and he included cycrimine,

procyclidine, and trihexyphenidyl hydrochloride salts in this group. In his report he described microcrystal tests and a number of colored spot tests. Vecerkova et al. (95) investigated the paper chromatographic behavior of several basic drugs which included procyclidine and trihexyphenidyl, and gave the corresponding R_f values. Gas chromatography has been applied to the identification of trihexyphenidyl (68) while Curry and Powell (96) studied paper chromatographic seperation of a number of alkaloidal or basic compounds which included trihexyphenidyl. Hayden et al. (97) published the infrared spectrum of both trihexyphenidyl hydrochloride and trihexyphenidyl base during a study of various U.S.P. and N.F. reference standard.

Methiodide, ethiodide and similiar derivatives have been prepared for these compounds and can serve as a useful aid to identification (29,98,99,100).

STATEMENT OF THE PROBLEM

Muscle relaxants have gained considerable popularity during the last fifteen years and have found a permanent place in the treatment of a wide variety of conditions. All of these preparations are potent systemic drugs and are intended for oral or parenteral administration. Since these compounds produce a pharmacological action through a systemic locus, their identification and differentiation is of concern to toxicologists and forensic chemists.

The literature survey revealed a complete lack of any adequate means of qualitative differentiation for this group of drugs and hence, there is an earnest need for a rapid and accurate means of identification.

Therefore, it is the purpose of this thesis to develop a comprehensive series of several physical reference criteria which can be utilized to positively identify these compounds in the least amount of time. The following techniques will be combined into the investigation of this problem.

(1) Preparation of derivatives of the parent compounds.

(2) Infrared studies of the parent compounds and their derivatives.

(3) Preparation of photomicrographs of several derivatives of the parent compounds.

EXPERIMENTAL

Apparatus: Fischer-Johns melting point apparatus (calibrated with melting point reference standards); 10 ml. burette, graduated to 0.05 ml.; Electromagnetic stirring apparatus; Heating mantle; Porcelain crucibles; Electric laboratory furnace (0-2000°C); Beckman IR-5A Infrared Spectrophotometer equipped with NaCl optics and adjusted according to the following qualitative settings: gain 7, aux. gain 3, damping 6, balance 4, S.B. 100% 9, and a pen speed of 16 minutes for full scale scanning from 2 to 16 minutes; Beckman #4240 Rectangular-Pellet Potassium Bromide Die; Carver Laboratory Press; Duo-Seal vacuum pump; and Wig-L-Bug amalgamator; Bausch & Lomb biological microscope equipped with a 5X eyepiece, 10X and 43X objective lenses, substage condenser with adjustable diaphragm, and a movable stage mounted on an Ortho-Illuminator with color and intensity adjustments at "D" and "3" respectively; Olympus PM-8 35 mm. microphotographic camera; Kodak Panatomic-X 35 mm. film; Microphotographic exposure meter, model 200 M (Photovolt Corp., N.Y.C.); Microscope slides, 75 x 25 mm.; Cover glasses, No. 1, 18 mm. sq.; 15 ml. amber dropper bottles; laboratory glassware; Metrohm Potentiograph model E 336.

Reagents and Solutions: Mephenesin; meprobamate; carisoprodal; phenyramidol hydrochloride; styramate; prenderol; methocarbamol; biperiden hydrochloride; procyclidine hydrochloride; trihexyphenidyl hydrochloride; cycrimine hydrochloride; acetone (A.C.S.); methanol (A.C.S.); picric acid (reagent grade); acetous perchloric acid, 0.05N (standardized against potassium acid phthalate, primary standard); crystal violet indicator solution (0.5% in glacial acetic acid, A.C.S.); methyl red indicator solution (0.1% in methanol, A.C.S.); toluene (A.C.S.); benzene (A.C.S.); methyl iodide (reagent grade); phosphorous pentoxide (A.C.S.); reinecke salt (reagent grade); sodium tetraphenylboron (reagent grade); chloroplatinic acid (A.C.S.);

chloroform (A.C.S.); xanthydrol (practical grade); 3-nitrophthalic anhydride (reagent grade); 3,5-dinitrobenzoyl chloride (reagent grade); benzhydol (reagent grade); acetic anhydride (A.C.S.); pyridine (A.C.S.); catalyst T-9 (this material is stannous 2-ethylhexanoate received from Metals and Thermit Corp., Hamilton, Ontario); pheny isocyanate (reagent grade); 1-naphthyl isocyanate (reagent grade); 2,4-dinitobenzenesulfenyl chloride (reagent grade); ethylene dichloride (reagent grade); petroleum ether (30-60°C, reagent grade); toluene-p-sulphonic acid (reagent grade); acetonitrile (certified); sulfuric acid (A.C.S.); sodium carbonate (A.C.S.) aqueous solution (2%); infrared quality potassium bromide (dried at 110°C for 48 hours); anhydrous sodium sulphate (B.P.C.); sodium hydroxide (A.C.S.) aqueous solution (10%); potassium iodide (A.C.S.); aqueous and alcoholic (95% ethanol, redistilled) solutions of: trihexyphenidyl hydrochloride 0.25%, 0.5%, 1.0%; procyclidine hydrochloride 0.25%, 0.5%, 1.0%; phenyramidol hydrochloride 0.25%, 0.5%, 1.0%; cycrimine hydrochloride 0.25%, 0.5%, 1.0%; biperiden hydrochloride 0.05%, 0.1%; aqueous reinecke salt solutions 0.1%, 0.25%, 0.5%, 1.0%; aqueous and alcoholic (95% ethanol, redistilled) picric acid (reagent grade) solutions 0.25%, 0.5%, 1.0%, and in acetone 0.5%, 1.0%; aqueous and alcoholic (95% ethanol, redistilled) styphnic acid solutions 0.16%, 0.32%, 0.64%, and 0.25%, 0.5%, 1.0% respectively; aqueous chloroplatinic acid solutions 0.25%, 0.5%, 1.0%; alcoholic (95% ethanol, redistilled) picrolonic acid (reagent grade) solutions 0.25%, 0.5%, and 1.0%. These solutions were stored in 15 ml. amber dropper bottles.

Elemental Analysis: All derivatives were submitted for carbon, hydrogen, and nitrogen elemental analysis to:

Dr. G. Weiler, Dr. F.B. Strauss
Microanalytical Laboratory
164 Banbury Road
Oxford, England.

A. FORMATION OF DERIVATIVES.

All of the purified derivatives were dried in a vacuum desiccator over phosphorous pentoxide at room temperature for twenty-four hours before the final melting point was taken on a Fischer-Johns melting point apparatus.

1) Phenyl Carbamates and 1-Naphthyl Carbamates

The Reed and Critchfield et al. (101) quantitative phenyl isocyanate method for determination of hydroxy equivalent weights was modified slightly and used as follows: about 250 mg. of the muscle relaxant was dissolved in a minimal amount of toluene. A slight excess of the calculated amount of isocyanate was added by pipette along with one drop of Catalyst T-9. The solution was warmed slightly and then set aside for about one or two hours until the crystals of carbamate came down. It was occasionally necessary to concentrate the solution on a water bath to help induce crystallization. Styramate was found to be only sparingly soluble in toluene. This compound was suspended in about 25 ml. of toluene, and the calculated amount of isocyanate and catalyst was added. The suspension was periodically heated and stirred over the next hour. It was then allowed to sit and react for about two hours at room temperature. The crystalline carbamate derivative was filtered (yield: ca. 64-95%), and recrystallized from 95% ethanol.

The following derivatives were prepared:

Mephenesin diphenylcarbamate, m.p. 121.0-122.5°C, yield 93.9%.
Formula: $C_{24}H_{24}N_2O_5$
Theoretical analysis: C 68.56%, H 5.75%, N 6.66%.
Found analysis: C 68.63%, H 5.67%, N 6.52%.

Metholcarbamol phenylcarbamate, m.p. 134.5-135.0°C, yield 85.6%.
Formula: $C_{18}H_{20}N_2O_6$
Theoretical analysis: C 59.99%, H 5.59%, N 7.77%.
Found analysis: C 59.69%, H 5.28%, N 7.70%.

DEP diphenylcarbamate, m.p. 136.0-137.0°C, yield 94.5%.
Formula: $C_{21}H_{26}N_2O_4$
Theoretical analysis: C 68.09%, H 7.07%, N 7.56%.
Found analysis: C 68.06%, H 7.11%, N 7.29%.

Styramate phenylcarbamate, m.p. 124.5-126.0°C, yield 81.7%.
Formula: $C_{16}H_{16}N_2O_4$
Theoretical analysis: C 64.01%, H 5.37%, N 9.33%.
Found analysis: C 64.16%, H 5.54%, N 9.40%.

Phenyramidol phenylcarbamate, m.p. 91.5-93.0°C, yield 87.5%.
Formula: $C_{20}H_{19}N_3O_2$
Theoretical analysis: C 72.05%, H 5.74%, N 12.61%.
Found analysis: C 71.96%, H 5.95%, N 12.31%.

Mephenesin 1-naphthylcarbamate, m.p. 108.0-109.0°C, yield 64.6%.
Formula: $C_{21}H_{21}NO_4$
Theoretical analysis: C 71.78%, H 6.02%, N 3.99%.
Found analysis: C 71.78%, H 5.91%, N 4.06%.

Mephenesin di-1-naphthylcarbamate, m.p. 182.5-184.0°C, yield 95.9%.
Formula: $C_{32}H_{28}N_2O_5$
Theoretical analysis: C 73.83%, H 5.42%, N 5.38%.
Found analysis: C 73.57%, H 5.61%, N 5.24%.

Methocarbamol 1-naphthylcarbamate, m.p. 87.0-88.5°C, yield 66.2%.
Formula: $C_{22}H_{22}N_2O_6$
Theoretical analysis: C 64.38%, H 5.40%, N 6.83%.
Found analysis: C 64.09%, H 5.15%, N 6.55%.

DEP di-1-naphthylcarbamate, m.p. 211.5-213.0°C, yield 92.5%.
Formula: $C_{29}H_{30}N_2O_4$
Theoretical analysis: C 74.02%, H 6.43%, N 5.95%.
Found analysis: C 74.04%, H 6.47%, N 5.98%.

Styramate 1-naphthylcarbamate, m.p. 133.0-134.0°C, yield 91.7%.
Formula: $C_{20}H_{18}N_2O_4$
Theoretical analysis: C 68.58%, H 5.18%, N 8.00%.
Found analysis: C 68.33%, H 5.24%, N 8.14%.

Phenyramidol 1-naphthylcarbamate, m.p. 152.5-153.5°C, yield 84.63%.
Formula: $C_{24}H_{21}N_3O_2$
Theoretical analysis: C 75.17%, H 5.52%, N 10.96%.
Found analysis: C 75.33%, H 5.45%, N 10.91%.

2) 3,5-Dinitrobenzoates

The general method of Cheronis and Entrikin (102) and Katz and Keeney (103) were combined and modified to give the following procedure: a minimal

amount of toluene was used to dissolve about 250 mg. of a muscle relaxant. A slight excess of the calculated amount of 3,5-dinitrobenzoyl chloride was dissolved in a minimal amount of toluene in a separate flask. The two solutions were poured together slowly with stirring; a slight excess of the calculated amount of pyridine, necessary to take up the HCl produced in the reaction, was added by pipette. The solution was mixed, tightly stoppered, and placed in a ½-inch deep, 40 C water bath for thirty minutes. At the end of the reaction time the toluene and the pyridine were removed under reduced pressure on a water bath. The residue was washed briefly with 5 ml. of 2.0% sodium carbonate and then with three 5 ml. portions of water. The residue was dissolved in a minimal amount of hot methanol or ethanol and cooled to facilitate crystallization. The crystalline benzoate was filtered (yield: ca. 42-47%), and recrystallized from 95% ethanol.

The following derivatives were prepared:

Mephenesin 3,5-dinitrobenzoate, m.p. 121.5-122.5°C, yield 42.3%.
Formula: $C_{17}H_{16}N_2O_8$
Theoretical analysis: C 54.26%, H 4.29%, N 7.45%.
Found analysis: C 54.08%, H 4.28%, N 7.28%.

Methocarbamol 3,5-dinitrobenzoate, m.p. 146.5-147.5°C, yield 46.5%.
Formula: $C_{18}H_{17}N_3O_{10}$
Theoretical analysis: C 49.66%, H 3.94%, N 9.65%.
Found analysis: C 49.58%, H 3.72%, N 9.33%.

DEP di-3,5-dinitrobenzoate, m.p. 142.5-143.5°C, yield 47.1%.
Formula: $C_{21}H_{20}N_4O_{12}$
Theoretical analysis: C 48.47%, H 3.87%, N 10.77%.
Found analysis: C 48.77%, H 4.14%, N 10.42%.

3) Acid 3-Nitrophthalates

The general procedure for the preparation of acid 3-nitrophthalates as outlined by Shriner *et al.* (104) was used for the preparation of these derivatives. A mixture of about 250 mg. of the muscle relaxant and a

calculated amount of 3-nitrophthalic anhydride, dissolved in pure, dry toluene was heated under a reflux condensor for a period of two to three hours. The toluene was removed under vacuo and the residue abstracted twice with 5 ml. of hot water, and then dissolved in minimal amount of hot 95% ethanol. The hot alcoholic solution was cooled to facilitate crystallization. The crystalline acid 3-nitrophthalates were filtered (yield: ca. 27-90%), and recrystallized from 95% ethanol.

The following derivatives were prepared:

Methocarbamol acid 3-nitrophthalate, m.p. 175.5-177.5°C, yield 26.8%.
Formula: $C_{19}H_{18}N_2O_{10}$
Theoretical analysis: C 52.54%, H 4.18%, N 6.45%.
Found analysis: C 52.34%, H 4.24%, N 6.26%.

DEP acid 3-nitrophthalate, m.p. 205.5-206.5°C, yield 89.5%.
Formula: $C_{15}H_{19}NO_7$
Theoretical analysis: C 55.38%, H 5.89%, N 4.31%.
Found analysis: C 55.22%, H 5.98%, N 4.23%.

Styramate acid 3-nitrophthalate, m.p. 187.0-189.5°C, yield 34.8%.
Formula: $C_{17}H_{14}N_2O_8$
Theoretical analysis: C 54.56%, H 3.77%, N 7.49%.
Found analysis: C 54.73%, H 3.86%, N 7.28%.

Phenyramidol acid 3-nitrophthalate, m.p. 194.5-195.5°C, yield 86.8%.
Formula: $C_{11}H_{13}NO_4$
Theoretical analysis: C 61.91%, H 4.21%, N 10.32%.
Found analysis: C 61.87%, H 4.39%, N 10.58%.

4) 2,4-Dinitrobenzenesulfenates

The procedure used by Kharasch et al. (105) was followed with slight modification. To a dry erylenmeyer flask was added about 5 to 10 ml. of ethylene chloride, and a calculated amount of 2,4-dinitrobenzenesulfenyl chloride. The mixture was swirled and heated gently to effect solution, then about 250 mg. of the muscle relaxant was added, the flask again swirled, and 0.25 ml. of pyridine added. A distinct deepening of the yellow color was observed as the pyridine dissolved in the solution. The reaction mixture was swirled and

allowed to stand for fifteen to thirty minutes to assure precipitation of any insoluble residue which may have formed. The mixture was filtered, and the residue (R_1) washed on the filter plate with a few ml. of hot ethylene chloride. The bright yellow R_1 (the amount of which varied somewhat with the muscle relaxant used, being from 10-15% of the sulfenate ester obtained) was discarded; and the filtrate aspirated to dryness (reduced pressure, room temperature). The residue from the filtrate was extracted with three 5 ml. portions of cold water and dissolved in a minimal amount of hot methanol or a hot solution of equal volumes of methanol and benzene. The solutions were cooled to facilitate crystallization. The crystalline sulfenate was filtered (yield: ca. 47-55%), recrystallized from methanol or a methanol-benzene mixture. In the instance of phenyramidol, the procedure as outlined by Wild (106) for the formation of amides of sulphenic acid was used. An ethereal solution of 2,4-dinitrobenzenesulfenyl chloride (one millimole) was added slowly, with stirring to an ethereal solution of phenyramidol base (two millimoles). Precipitation of the amine hydrochloride was filtered, washed with ether, and discarded. The ethereal filtrate was reduced under vacuum on a water bath and the residue was recrystallized from ethyl alcohol (95%).

The following derivatives were prepared:

Mephenesin di-2,4-dinitrobenzenesulfenate, m.p. 158.5-159.5°C, yield 55.4%.
Formula: $C_{22}H_{18}N_4O_{11}S_2$
Theoretical analysis: C 45.68%, H 3.14%, N 9.69%.
Found analysis: C 45.49%, H 2.92%, N 9.54%.

Methocarbamol 2,4-dinitrobenzenesulfenate, m.p. 199.5-201.0°C, yield 49.0%.
Formula: $C_{17}H_{17}N_3O_9S$
Theoretical analysis: C 46.47%, H 3.90%, N 9.56%.
Found analysis: C 46.74%, H 3.93%, N 9.80%.

Styramate 2,4-dinitrobenzenesulfenate, m.p. 181.0-182.5°C, yield 52.2%.
Formula: $C_{15}H_{13}N_3O_7S$
Theoretical analysis: C 47.50%, H 3.46%, N 11.08%.
Found analysis: C 47.55%, H 3.54%, N 10.88%.

Phenyramidol 2,4-dinitrobenzenesulfenate, m.p. 111.5-113.0°C (decomp.), yield 47.7%.
Formula: $C_{19}H_{16}N_4O_5S$
Theoretical analysis: C 55.32%, H 3.91%, N 13.58%.
Found analysis: C 55.46%, H 4.16%, N 13.58%.

5) Xanthyl Derivatives

The general procedure of Dechene (74) was modified and used as follows: good yield of derivative were obtained by dissolving about 200 mg. of muscle relaxant and a slight excess of the calculated amount of xanthydrol in glacial acetic acid, and allowing the solution to stand for ten hours or more at room temperature. The crystals of the derivative generally precipitated in this period of time but in some instances the solution had to be concentrated under vacuum, and then cooled to induce crystallization. The crystalline xanthyl derivative was filtered (yield: ca. 82-96%), washed well with distilled water, and recrystallized from hot methanol.

Xanthyl derivatives prepared:

Xanthyl methocarbamol, m.p. 137.5-138.0°C, yield 86.4%.
Formula: $C_{24}H_{23}NO_6$
Theoretical analysis: C 68.40%, H 5.50%, N 3.32%.
Found analysis: C 68.68%, H 5.78%, N 3.09%.

Xanthyl styramate, m.p. 165.5-167.0°C, yield 84.6%.
Formula: $C_{22}H_{19}NO_4$
Theoretical analysis: C 73.14%, H 5.30%, N 3.88%.
Found analysis: C 73.19%, H 5.48%, N 3.77%.

Xanthyl carisoprodal, m.p. 141.0-142.5°C, yield 82.3%.
Formula: $C_{25}H_{33}O_5N_2$
Theoretical analysis: C 68.16%, H 7.55%, N 6.36%.
Found analysis: C 68.20%, H 7.36%, N 6.48%.

Dixanthyl meprobamate, m.p. 188.0-189.0°C, yield 95.6%.
Formula: $C_{35}H_{34}N_2O_6$
Theoretical analysis: C 72.65%, H 5.92%, N 4.84%.
Found analysis: C 72.86%, H 6.09%, N 4.56%.

6) Diphenylmethyl Derivatives

The general procedure of the characterization of amides by the formation of diphenylmethyl derivatives as outlined by Cheeseman and Poller (107), was used for the preparation of these derivatives. A solution of the muscle relaxant (250 mg.), and an equal molar amount of benzhydrol and toluene-p-sulphonic acid were dissolved in about 5 ml. of glacial acetic acid and boiled under reflux for about one hour. As the mixture was heated a transient blue color was produced. The hot mixture was then poured slowly and with stirring into ten volumes of water and in all instances the crude derivative precipitated as an oily mass. The supernatant aqueous material was later poured off and the oily mass in the bottom of the flask was dissolved in hot ethanol (95%). This alcoholic solution was cooled and the crystals were filtered and recrystalized from hot ethanol (95%). Yields ranged from 75-82%.

Diphenylmethyl derivatives prepared were:

N,N-Di(diphenylmethyl)meprobamate, m.p. 108.0-109.0°C, yield 82.3%.
Formula: $C_{35}H_{38}N_2O_4$
Theoretical analysis: C 76.34%, H 6.96%, N 5.09%.
Found analysis: C 76.04%, H 7.18%, N 4.98%.

N-Diphenylmethyl carisoprodal, m.p. 95.5-97.0°C, yield 75.6%.
Formula: $C_{25}H_{35}N_2O_4$
Theoretical analysis: C 70.40%, H 8.27%, N 6.57%.
Found analysis: C 70.43%, H 7.99%, N 6.40%.

7) Acetyl Derivatives

The general procedure as outlined in the B.P. (42) and U.S.P. (41) for the preparation of the diacetyl derivative of meprobamate was used for the preparation of these derivatives. The muscle relaxant (500 mg.) was mixed with 1 ml. of acetic anhydride, and one drop of sulfuric acid was added. The mixture was stirred to effect solution and then allowed to stand for thirty minutes at room temperature.

The solution was poured slowly into 50 ml. of water while stirring the mixture vigorously, and then allowed to crystallize. The crystalline derivatives were filtered (yield: ca. 86-95%), washed well with water, and then recrystallized from ethanol/water.

Acetylation products prepared:

N-Acetyl-O-Acetyl methocarbamol, m.p. 84.0-85.0°C, yield 94.1%.
Formula: $C_{15}H_{19}NO_7$
Theoretical analysis: C 55.38%, H 5.89%, N 4.31%.
Found analysis: C 54.98%, H 5.97%, N 4.68%.

N-Acetyl-O-Acetyl styramate, m.p. 96.5-98.0°C, yield 92.6%.
Formula: $C_{13}H_{15}NO_5$
Theoretical analysis: C 58.88%, H 5.70%, N 5.28%.
Found analysis: C 59.02%, H 5.75%, N 5.53%.

N,N-Diacetyl meprobamate, m.p. 127.5-128.5°C, yield 94.7%.
Formula: $C_{13}H_{22}N_2O_6$
Theoretical analysis: C 51.65%, H 7.34%, N 9.27%.
Found analysis: C 51.59%, H 7.25%, N 9.40%.

N-Acetyl carisoprodal, m.p. 87.0-88.5°C, yield 86.8%.
Formula: $C_{14}H_{27}N_2O_5$
Theoretical analysis: C 55.61%, H 9.00%, N 9.27%.
Found analysis: C 55.66%, H 8.79%, N 9.38%.

8) Preparation and Assay of Picrates

Preparation: The general procedure for the preparation of picrates as outlined by Shriner et al. (104) was modified slightly and used as follows: a solution of 250 mg. of a aminoalcohol salt dissolved in 10 ml. of 95% ethanol was poured into 10 ml. of a saturated solution of picric acid and the mixture heated to boiling and cooled. It was occasionally necessary to concentrate the solution on a water bath to induce crystallization upon cooling. The crystalline picrate was filtered (yield: ca. 62-82%), and recrystallized from 95% ethanol.

Assay: The picrates were titrated in glacial acetic acid according to the following technique for picrates by Clark and Wang (108): about 1 meq. of the picrate, accurately weighed, was dissolved in 5 ml. of acetone and 45 ml. of glacial acetic acid with gentle heat when

necessary. Two drops of crystal violet indicator solution were added, and the solution was titrated to a blue end point with 0.05N acetous perchloric acid while stirred electromagnetically. A blank determination on the solvent system required 0.05 ml. of the titrant.

The following picrates were prepared:

Cycrimine picrate, m.p. 145.0-146.5°C, yield 81.4%, purity by titrimetry 99.32%.
Formula: $C_{25}H_{32}N_4O_8$
Theoretical analysis: C 58.14%, H 6.25%, N 10.85%.
Found analysis: C 57.98%, H 6.14%, N 10.68%.

Procyclidine picrate, m.p. 63.5-65.0°C, yield 62.5%, purity by titrimetry 99.52%.
Formula: $C_{25}H_{32}N_4O_8$
Theoretical analysis: C 58.14%, H 6.25%, N 10.85%.
Found analysis: C 58.16%, H 6.26%, N 10.57%.

Phenyramidol picrate, m.p. 153.5-154.5°C, yield 78.7%, purity by titrimetry 99.89%.
Formula: $C_{19}H_{17}N_5O_8$
Theoretical analysis: C 51.47%, H 3.87%, N 15.80%.
Found analysis: C 51.32%, H 3.63%, N 15.49%.

9) Preparation and Assay of the Reineckates

Preparation: The procedure of Chatten and Levi (109) was modified and used as follows: to 250 mg. of the aminoalcohol salt dissolved in a minimal amount of water, a slight excess of a calculated amount of aqueous reinecke salt solution was added slowly with constant stirring. The resulting precipitated mass was allowed to stand fifteen minutes at room temperature before the amorphous or crystalline precipitate was filtered off (yield: *ca.* 85-91%), and washed with cold water until the filtrate was clear. The derivative was recrystallized by adding approximately 5 to 10 ml. of methanol and sufficient acetone dropwise to solubilize the derivative if it had not already dissolved. Water was then added dropwise with agitation until a turbidity was seen to persist. The vessel was cooled in an ice bath and the resulting crystalline material separated by filtration. It must be noted that

the preparation and recrystallization of these compounds was carried out without the aid of heat. The derivatives were dried in vacuo over phosphorous pentoxide, and analyzed by a gravimetric method for chromium content.

Assay: The purity of the reinecke derivatives was determined gravimetrically by an ashing procedure. About 50 mg. of the reineckate, accurately weighed, was heated at 1500°C for one hour in a porcelain crucible. The resulting Cr_2O_3 residue was accurately weighed and related to the chromium content of the original reineckate.

The following reineckates were prepared and the results of the gravimetric analyses are given:

Cycrimine reineckate, m.p. 154.0-158.0°C (decomp.), yield 86.5%.
Formula: $C_{23}H_{38}CrN_7O_2S_4$
Theoretical analysis: C 45.52%, H 5.98%, Cr 8.57%, N 16.16%.
Found analysis: C 45.72%, H 6.20%, Cr 8.51%, N 15.89%.

Procyclidine reineckate, m.p. 149.0-154.0°C (decomp.), yield 84.6%.
Formula: $C_{23}H_{38}CrN_7O_2S_4$
Theoretical analysis: C 45.52%, H 5.98%, Cr 8.57%, N 16.16%.
Found Analysis: C 45.29%, H 5.96%, Cr 8.68%, N 15.93%.

Trihexyphenidyl reineckate, m.p. 159.0-162.0°C (decomp.), yield 86.2%.
Formula: $C_{24}H_{40}CrN_7O_2S_4$
Theoretical analysis: C 46.42%, H 6.17%, Cr 8.38%, N 15.79%.
Found analysis: C 46.48%, H 6.32%, Cr 8.29%, N 15.65%.

Biperiden reineckate, m.p. 160.0-164.0°C (decomp.), yield 88.1%.
Formula: $C_{25}H_{88}CrN_7O_2S_4$
Theoretical analysis: C 47.60%, H 5.75%, Cr 8.24%, N 15.54%.
Found analysis: C 47.25%, H 5.71%, Cr 8.16%, N 15.35%.

Phenyramidol reineckate, m.p. 156.0-158.0°C (decomp.), yield 91.0%.
Formula: $C_{17}H_{23}CrN_8O_2S_4$
Theoretical analysis: C 38.26%, H 3.97%, Cr 9.75%, N 21.00%.
Found analysis: C 38.15%, H 4.20%, Cr 9.68%, N 20.77%.

10) Preparation and Assay of the Tetraphenylborates (TPB's)*

Preparation: A slightly modified technique of Koehler and Feldmann (110) was used for the preparation of these derivatives. About 250 mg.

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* "Tetraphenylborates" may hereafter be referred to as "TPB's".

of the aminoalcohol salt was dissolved in a minimal amount of water. The resultant solution when tested with universal indicator paper was shown to have an acidic pH in all instances. A slight excess of sodium tetraphenylboron was dissolved in a minimal amount of water in a separate vessel. The two solutions were added slowly together with constant stirring, and the mixture was then allowed to stand at room temperature for twenty minutes. The precipitate was filtered and washed well with water until the filtrate was clear (yield: ca. 88-94%). Recrystallization was from methanol or methanol/acetone by adding water dropwise with agitation until a turbidity persisted, and then cooled. The resultant derivatives were dried in vacuo over phosphorous pentoxide, and titrated in nonaqueous media.

Assay: A nonaqueous titration technique for local anesthetic tetraphenylborates as outlined by Chatten et al. (111) was used. To 50 mg. of an aminoalcohol TPB, accurately weighed, dissolved in 5 ml. of acetone and 45 ml. of glacial acetic acid, two drops of crystal violet indicator solution was added. The solution was titrated with 0.05N acetous perchloric acid to a blue end point with constant electromagnetic stirring.

The tetraphenylborate derivatives were prepared and the results of the nonaqueous titrations are included in the following data.

Cycrimine TPB, m.p. 157.5-159.0°C, yield 90.2%, purity by titrimetry 99.49%.
Formula: $C_{43}H_{50}BNO$
Theoretical analysis: C 85.00%, H 8.29%, N 2.31%.
Found analysis: C 84.76%, H 8.30%, N 2.12%.

Procyclidine TPB, m.p. 148.0-150.0°C, yield 94.1%, purity by titrimetry 99.27%.
Formula: $C_{43}H_{50}BNO$
Theoretical analysis: C 85.00%, H 8.29%, N 2.31%.
Found analysis: C 84.55%, H 8.43%, N 2.15%.

Trihexyphenidyl TPB, m.p. 157.0-159.0°C, yield 88.0%, purity by titrimetry 100.69%.
Formula: $C_{44}H_{52}BNO$
Theoretical analysis: C 85.00%, H 8.41%, N 2.25%.
Found analysis: C 84.72%, H 8.75%, N 2.05%.

Biperiden TPB, m.p. 208.0-209.5°C, yield 89.3%, purity by titrimetry 99.72%.
Formula: $C_{45}H_{50}BNO$
Theoretical analysis: C 85.57%, H 7.98%, N 2.22%.
Found analysis: C 85.26%, H 8.19%, N 2.17%.

Phenyramidol TPB, m.p. 124.0-126.0°C, yield 91.6%, purity by titrimetry 100.32%.
Formula: $C_{37}H_{35}BN_2O$
Theoretical analysis: C 83.14%, H 6.60%, N 5.24%.
Found analysis: C 82.98%, H 6.63%, N 5.46%.

11) Methiodide Derivatives

The procedures for the preparation of the methiodide derivatives as outlined by Cheronis and Entrikin (102) and Adamson *et al.* (29) were combined and modified to the following technique: about 250 mg. of the amino muscle relaxant salt was dissolved in a minimal amount of water in a separatory funnel. A slight excess of 10 per cent sodium hydroxide solution was added and shaken well. The free base was extracted with three 5 ml. portions of ether, and the combined ethereal extractions were dried with anhydrous sodium sulfate. A slight excess of methyl iodide was added and the solution refluxed for five minutes. The solution was then allowed to sit at room temperature until the crystals came down. The cystalline derivative was filtered off (yield: *ca.* 65-82%), washed with ether, and recrystallized from isopropanol or acetone/ether. The methyl iodide derivative was dried in vacuo over phosphorous pentoxide for twenty-four hours.

The following methiodides were prepared:

Cycrimine methiodide, m.p. 145.0-146.0°C, yield 71.6%.
Formula: $C_{20}H_{32}INO$
Theoretical analysis: C 55.95%, H 7.51%, N 3.26%.
Found analysis: C 56.06%, H 7.45%, N 3.28%.

Procyclidine methiodide, m.p. 204.0-205.0°C, yield 68.3%.
Formula: $C_{20}H_{32}INO$
Theoretical analysis: C 55.95%, H 7.51%, N 3.26%.
Found analysis: C 55.68%, H 7.73%, N 3.43%.

Trihexyphenidyl methiodide, m.p. 208.0-208.5°C, yield 79.8%.
Formula: $C_{21}H_{34}INO$
Theoretical analysis: C 56.88%, H 7.73%, N 3.16%.
Found analysis: C 56.89%, H 7.85%, N 3.41%.

Biperiden methiodide, m.p. 207.0-208.0°C, yield 65.7%.
Formula: $C_{22}H_{32}INO$
Theoretical analysis: C 58.28%, H 7.11%, N 3.09%.
Found analysis: C 58.31%, H 7.17%, N 3.22%.

Phenyramidol methiodide, m.p. 165.5-166.5°C, yield 82.4%.
Formula: $C_{14}H_{17}IN_2O$
Theoretical analysis: C 47.21%, H 4.81%, N 7.87%.
Found analysis: C 47.27%, H 4.85%, N 7.98%.

12) Chloroplatinate Derivatives

The method outlined by Wild (106) for the preparation of chloroplatinic acid derivatives was modified and adapted as follows: approximately 250 mg. of the amino muscle relaxant salt was dissolved in a minimal amount of water. A slight excess of a equal molar amount of chloroplatinic acid was dissolved in a minimal amount of water in a separate flask. The two solutions were added together slowly with vigorous stirring. Biperiden hydrochloride, being almost insoluble in water was dissolved in a minimal amount of methanol, to which a slight excess of chloroplatinic acid, dissolved in water, was added with stirring. The mixtures were allowed to stand twenty minutes in an ice bath and then filtered (yield: ca. 87-98%). The finely crystalline precipitate was purified by washing well with cold water until the filtrate was clear.

The following chloroplatinate derivatives were prepared:

Cyrimine chloroplatinate, m.p. 179.0-182.0°C (decomp.), yield 95.6%.
Formula: $C_{38}H_{60}Cl_6N_2O_2Pt$
Theoretical analysis: C 46.36%, H 6.14%, N 2.85%.
Found analysis: C 46.27%, H 6.14%, N 3.03%.

Procyclidine chloroplatinate, m.p. 199.0-203.0°C (decomp.), yield 97.3%.
Formula: $C_{38}H_{60}Cl_6N_2O_2Pt$
Theoretical analysis: C 46.36%, H 6.14%, N 2.85%.
Found analysis: C 46.44%, H 6.29%, N 2.88%.

Trihexyphenidyl chloroplatinate, m.p. 132.0-137.0°C (decomp.), yield 98.4%.
Formula: $C_{40}H_{64}Cl_6N_2O_2Pt$
Theoretical analysis: C 47.44%, H 6.17%, N 2.77%.
Found analysis: C 47.35%, H 6.66%, N 2.75%.

Biperiden chloroplatinate, m.p. 142.0-147.0°C (decomp.), yield 96.7%.
Formula: $C_{42}H_{60}Cl_6N_2O_2Pt$
Theoretical analysis: C 48.85%, H 5.86%, N 2.71%.
Found analysis: C 48.61%, H 6.00%, N 2.59%.

Phenyramidol chloroplatinate, m.p. 156.0-160.0°C (decomp.), yield 87.9%.
Formula: $C_{26}H_{30}Cl_6N_4O_2Pt$
Theoretical analysis: C 37.25%, H 3.61%, N 6.68%.
Found analysis: C 37.35%, H 3.65%, N 6.74%.

B. PREPARATION OF INFRARED SPECTRA.

Procedure: The infrared spectra were obtained from 2 to 16 microns using a Beckman IR-5A infrared spectrophotometer with the use of KBr disks. Approximately 1 mg. samples of dried, purified derivatives (or a muscle relaxant recrystallized from methanol) were mixed with sufficient dried KBr (infrared quality) to make a 300 mg. powder mixture in a steel capsule mounted on a Wig-L-Bug (112). The finely powdered mixture was transferred to a Beckman rectangular-pellet die which was then subjected to vacuum, and compressed at 18,000 lbs./sq.in. in a Carver laboratory hydraulic press. After several minutes, the vacuum was released, and the infrared spectrum of the resulting pellet was recorded. A KBr blank was placed in the reference beam to compensate for any residual or adsorbed moisture in the KBr.

C. PREPARATION OF PHOTOMICROGRAPHS.

Procedure: The general method of Chatten and Levi (109) was modified as follows: one drop of a 0.25, 0.5, or 1.0 % aqueous solution of an amine muscle relaxant salt was placed on a microscope slide. One drop of a reagent solution was added, mixed well, and covered with a cover glass. For the potassium iodide test, one drop of an aqueous solution of the parent compound was placed on a microscope slide and a few particles of finely ground potassium iodide were sprinkled over the surface of the drop.

In all instances the time of crystal formation was noted, and photomicrographs were taken before the slide became dry.

RESULTS AND DISCUSSION

A. DERIVATIZATION.

In addition to determining the individual specific physical properties of any given organic compound, the preparation of derivatives is one of the most reliable and conclusive methods for qualitative identification. The derivatives prepared from the muscle relaxants fall into two categories; (i) alcoholic group derivatives and (ii) amino group derivatives.

1) Phenyl and 1-Naphthyl Carbamates

Most alcohols react readily with isocyanates, according to the following general equation, to give an almost theoretical yield of the corresponding urethane or carbamate (106).

$$R\text{-}OH + R'\text{-}N{=}C{=}O \longrightarrow R'\text{-}NH\text{-}COOR$$

The alcohol should be virtually anhydrous, as water reacts with the isocyanate reagent forming the corresponding symmetrical diaryl urea. A substituted, unstable, carbamic acid is formed first and breaks down to give a primary amino compound which reacts with a second molecule of the isocyanate to give the final product.

$$H_2O + R\text{-}N{=}C{=}O \longrightarrow (R\text{-}NH\text{-}COOH) \longrightarrow R\text{-}NH_2 + CO_2$$

$$R\text{-}NH_2 + R\text{-}N{=}C{=}O \longrightarrow R\text{-}NH\text{-}CO\text{-}NH\text{-}R$$

Isocyanates usually are restricted to the characterization of primary and secondary alcohols, as the majority of tertiary alcohols are dehydrated to olefins, or do not react, under the conditions used.

Since many of the muscle relaxants are 1,2 or 1,3-propanediol derivatives most of them contain at least one free primary or secondary hydroxyl group. A review of the literature has revealed that the carbamate ester has been prepared for many of these compounds (113,114,43) but the phenyl carbamate and 1-naphthyl carbamate esters have not been synthesized.

Table I. Phenyl Carbamate Derivatives

Parent Compound	Melting Point °C (corr.)	Type of Derivative (alcohol:reagent)	Molecular Weight
Mephenesin	121.0-122.5	1:2	420.46
DEP	136.0-137.0	1:2	370.47
Methocarbamol	134.5-135.0	1:1	360.37
Styramate	124.5-126.0	1:1	300.24
Phenyramidol	91.5-93.0	1:1	333.40

Table II. 1-Naphthyl Carbamate Derivatives

Parent Compound	Melting Point °C (corr.)	Type of Derivative (alcohol:reagent)	Molecular Weight
Mephenesin	108.0-109.0	1:1	351.39
Mephenesin	182.5-184.0	1:2	520.58
DEP	211.5-213.0	1:2	470.55
Methocarbamol	87.0-88.5	1:1	410.41
Styramate	133.0-134.0	1:1	350.295
Phenyramidol	152.5-153.5	1:1	383.46

It was possible to obtain a phenyl or 1-naphthyl carbamate ester of each of the compounds containing a primary or secondary alcoholic group. Generally the carbamates proved to be a highly desirable derivative for characterizing the muscle relaxants described in Table I and Table II, since good yields and sharp,well distributed melting points were obtained.

The mono-phenylcarbamate derivative of mephenesin would not separate from the toluene mixture,even upon cooling or reducing the solution under vacuum. Finally the solution was reduced to dryness and the resulting oily mass was washed well with petroleum ether and then dissolved in

a minimal amount of hot ethanol (95%). It was then cooled slowly and the derivative came down as an oily mass, which continued to separate on repeated crystallization attempts with methanol and acetone, and we were unable to obtain this derivative in a crystalline form.

The isocyanate-alcohol reaction was catalyzed by stannous 2-ethylhexanoate (obtained from Metals and Thermit Corp., Hamilton, Ontario). The catalyst has been used successfully by Reed et al. (101) during a study of a pheny isocyanate method for the determination of the hydroxyl equivalent weight of polyoxyalkylene compounds. Smith (115) has made extensive catalytic investigations for the formation of urethanes using this metal salt catalyst,and has found that the catalyst is applicable to most isocyanate-alcohol reactions.

Primary and secondary amines can be characterized by the formation of substituted ureas through a reaction with some aryl isocyanate. Since phenyramidol contains both a secondary hydroxyl and secondary amine functional group, it is possible that the aryl isocyanates reacted with either the hydroxyl or the amine functional group. The elemental analyses for the carbamate derivatives of this compound showed the formation of a 1:1 product.

2) 3,5-Dinitrobenzoates

Alcohols can be esterified by warming gently with a slight excess of an acid chloride in the presence of dilute sodium hydroxide or pyridine. The following equation illustrates the formation of 3,5-dinitrobenzoates using the reagent 3,5-dinitrobenzoyl chloride.

$$(NO_2)_2C_6H_3COCl \ + \ R\text{-}OH \longrightarrow (NO_2)_2C_6H_3COOR \ + \ HCl$$

The method often fails with tertiary alcohols, as olefins are formed under most experimental conditions, and even with primary and secondary alcohols

there are great differences in the reactivity of the hydroxyl group. The yield of ester from a tertiary alcohol is generally insufficient for ordinary methods of purification and melting-point determination.

Katz and Keeney (103) found that low concentrations of pyridine were required for maximum ester formation, while high pyridine concentrations depressed the yield. Thus only a slight excess of the calculated amount of pyridine required to combine with the HCl produced in the reaction was added. The product of the reaction was extracted briefly with dilute sodium carbonate solution to remove any resulting 3,5-dinitrobenzoic acid or its anhydride.

Many of the derivatives (i.e., mephenesin di-3,5-dinitrobenzoate, styramate 3,5-dinitrobenzoate, and phenyramidol 3,5-dinitobenzoate) could not be crystallized upon repeated attempts in various solvents (methanol, ethanol, acetone), and an oily mass was obtained in all instances. However, the 3,5-dinitrobenzoate derivatives proved to be satisfactory for some of the muscle relaxants, as shown in Table III. High yields of derivative were not obtained but usually only one recrystallization was necessary to obtain a purified product.

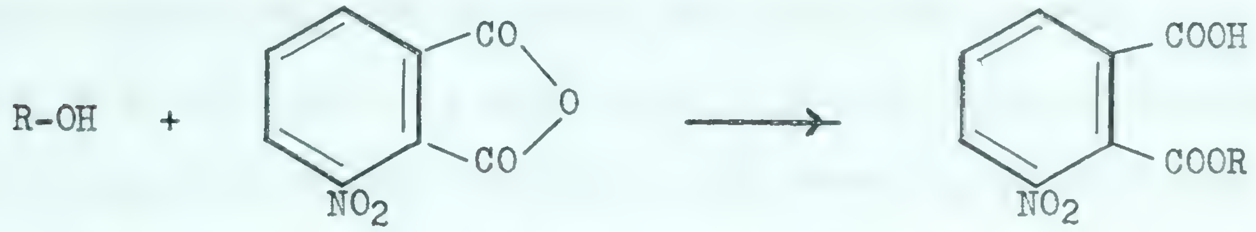
Table III. 3,5-Dinitrobenzoate Derivatives

Parent Compound	Color	Melting Point °C (corr.)	Type of Derivative (alcohol:reagent)	Molecular Weight
Mephenesin	DY	121.5-122.5	1:1	376.31
DEP	VPY	142.5-143.5	1:2	520.41
Methocarbamol	PY	146.5-147.5	1:1	435.35

Abbreviations: DY = dark yellow; VPY = very pale yellow; PY = pale yellow.

3) Acid 3-Nitrophthalates

The use of 3-nitrophthalic anhydride as a reagent for the identification of alcohols was suggested by Nicolet and Sachs (116). Investigations by Ashdown and Monier (117) showed that the 2-mono-alkyl esters were the principal product and that the 1-mono-alkyl esters were eliminated by recrystallization. Interaction of an alcohol with 3-nitrophthalic anhydride to produce the corresponding ester is illustrated below:



The acid 3-nitrophthalate esters were prepared for all the muscle relaxants containing a primary or a secondary hydroxyl group with the exception of mephenesin. Mephenesin (mono and di) acid 3-nitrophthalate esters could not be isolated as crystalline products. In each instance, repeated crystallization attempts from various solvents (ethanol,methanol,acetone) resulted in a gummy oily mass.

Although yields varied greatly it was possible to obtain a workable amount of the derivative with the compounds described in Table IV.

Table IV. Acid 3-Nitrophthalate Derivatives

Parent Compound	Color	Melting Point °C (corr.)	Type of Derivative (alcohol:reagent)	Molecular Weight
DEP	W	205.5-206.5	1:1	325.33
Methocarbamol	PY	175.5-177.5	1:1	434.37
Styramate	W	187.0-189.5	1:1	374.23
Phenyramidol	W	194.5-195.5	1:1	407.39

Abbreviations: W = white; PY = pale yellow.

Since 3-nitrophthalic anhydride can react with primary or secondary amines as well as with the alcoholic functional group, the phenyramidol acid 3-nitrophthalate ester proved to be of some interest. Elemental analysis of the derivative showed that the product was the 1:1 compound. In order to ascertain whether the reagent had attacked the hydroxyl or the amino group, phenyramidol base and the derivative were potentiometrically titrated in nonaqueous media according to the procedure of Clair and Chatten (118). In each instance the potentiometric titration curves revealed only one end point, and thus both compounds were titrating on a 1:1 basis. If the 2-amino group had been substituted, the relative basicity of the derivative would have changed from that of the parent phenyramidol base, and no potentiometric end point would have resulted.

4) <u>2,4-Dinitrobenzenesulfenate Derivatives</u>

The reagent 2,4-dinitrobenzenesulfenyl chloride reacts with a series of primary, secondary, and tertiary alcohols in accord with the equation:

$$(NO_2)_2C_6H_3SCl \ + \ R\text{-}OH \longrightarrow (NO_2)_2C_6H_3SOR \ + \ HCl$$

Pyridine greatly facilitates formation of the 2,4-dinitrobenzenesulfenate esters, thereby permitting a general technique for the characterization of the alcoholic group (105). The obvious role of pyridine in these reactions would appear to be neutralization of hydrogen chloride, thereby preventing reversal,but preliminary work on the reactions of sulfenyl halides with tertiary amines (with and without alcohols present) suggests that the role of pyridine may be more complex (119). Kharash et al.(105) have found that the sulfenyl halides may react with dry pyridine to form disulphides and has proposed a mechanism for such disulphide formation. These workers suspect that a reactive pyridinium salt (ArSPyCl) may be involved as the first step. Reactions of such an intermediate with the

sulfenyl chloride might yield disulphide:

$$\mathrm{ArSPyCl} + \mathrm{ArSCl} \longrightarrow \mathrm{ArSSAr} + \mathrm{PyCl_2} \quad (?)$$

Reaction with the alcohol could lead to the sulfenate ester:

$$\mathrm{ArSPyCl} + \mathrm{R{-}OH} \longrightarrow \mathrm{ArSOR} + \mathrm{Py\ HCl} \quad (?)$$

However, the use of pyridine fosters rapid completion of the reactions and permits relatively good yields.

The benzenesulfenate esters could be prepared for the majority of the muscle relaxants containing a primary or secondary hydroxyl group. Mephenesin mono-2,4-dinitrobenzenesulfenate and DEP di-2,4-dinitrobenzenesulfenate esters were the only anomalous derivatives that could not be isolated in a crystalline state. Repeated crystallization attempts were made in various solvent systems (ethanol,methanol,acetone,methanol: benzene) but an oily residue was obtained with each trial.

Since sulfenyl halides can react with primary and secondary amines as well as with the alcoholic function, the phenyramidol derivative was treated in exactly the same manner as the previously mentioned phenyramidol acid 3-nitrophthalate. The derivative was titrated potentiometrically in nonaqueous media and found to titrate 1:1, thus proving that the sulfenyl halide had not attacked the 2-amino but rather the hydroxyl group.

2,4-Dinitrobenzenesulfenyl chloride proved to be a good reagent for the preparation of derivatives for those agents listed in Table V.

Table V. 2,4-Dinitrobenzenesulfenate Derivatives

Parent Compound	Melting Point °C (corr.)	Type of Derivative (alcohol:reagent)	Molecular Weight
Mephenesin	158.5-159.5 (a)	1:2	578.51
Methocarbamol	199.5-201.0 (a)	1:1	439.40
Styramate	181.0-182.5 (b)	1:1	379.26

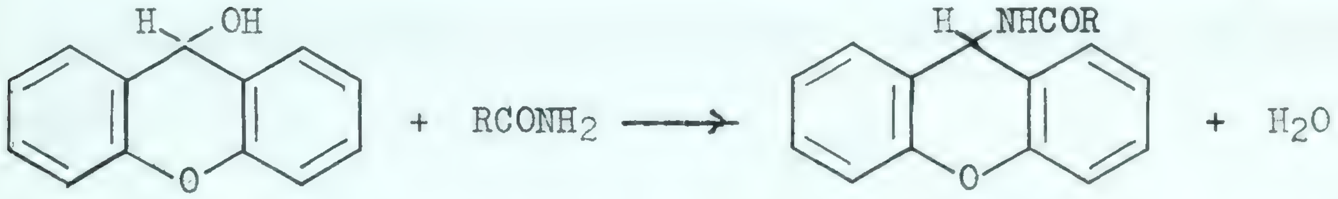
Table V. - Continued

Parent Compound	Melting Point °C (corr.)	Type of Derivative (alcohol:reagent)	Molecular Weight
phenyramidol	111.5-113.0 (D)(b)	1:1	412.52

Abbreviations: D = decomposition.
(a) Recrystallized from an equal volume mixture of methanol and benzene.
(b) Recrystallized from methanol/water.

5) Xanthyl Derivatives

Primary amides condense rapidly with xanthydrol in solution to form solids useful for characterization (120,121). The reaction of amides with xanthydrol takes place according to the following general equation:



Roth and others (72) have characterized meprobamate by means of its dixanthyl derivative and report a melting point of 182°C, while Dechene (74) has reported the same derivative with a higher melting point of 188 to 189°C.

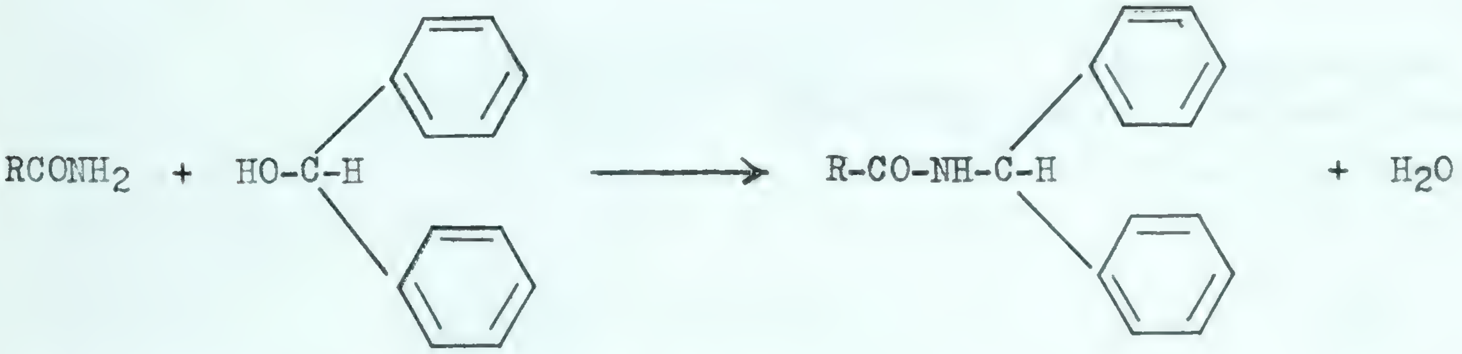
Since the aforementioned workers have successfully applied xanthydrol to the characterization of various amides and carbamates, it was decided to apply this reagent to the identification of various carbamate muscle relaxants. Derivatives prepared were easily purified, obtained in good yields, had well distributed melting points, and were generally very satisfactory with the major disadvantage being their time of formation (ten hours). Table VI briefly summarizes the xanthyl derivatives prepared and lists the corresponding melting points.

Table VI. Xanthyl Derivatives

Parent Compound	Melting Point, °C Found (corr.)	Melting Point, °C Literature	Type of Derivative (carbamate:reagent)
Methocarbamol	137.5-138.0		1:1
Styramate	165.5-167.0		1:1
Carisoprodal	141.0-142.5		1:1
Meprobamate	188.0-189.0	182.0 (72) 188.0-189.0 (74)	1:2

6) Diphenylmethyl Derivatives

The reaction of diphenylmethanol (benzhydrol) with amides in the presence of toluene-p-sulphonic acid has been shown to be applicable and yield satisfactory derivatives with an extensive range of primary amides (107). A general equation to illustrate such a reaction would be described as follows:



Since all the carbamate muscle relaxants contain the amide grouping, it was decided to use the benzhydrol reagent in an attempt to prepare derivatives of this moiety.

Diphenylmethyl derivatives of styramate and methocarbamol could not be isolated in a crystalline form, although repeated crystallization attempts were made from various solvents (methanol,ethanol,benzene,acetone).

Good yields of characteristic derivative could be obtained for the carbamate muscle relaxants outlined in Table VII, and melting points are sufficiently sharp to allow them to be used to identify these compounds.

Table VII. Diphenylmethyl Derivatives

Parent Compound	Melting Point C (corr.)	Type of Derivative (carbamate:reagent)	Molecular Weight
Meprobamate	108.0-109.0	1:2	550.678
Carisoprodal	95.5-97.0	1:1	426.54

7) Acetyl Derivatives

Acetic anhydride has been used by the B.P. (42) and the U.S.P. (41) to prepare the diacetyl derivative of the carbamate moieties found in meprobamate. In an effort to expand this approach, it was decided to prepare the acetyl derivative of some of the muscle relaxants containing the carbamate group.

All of the carbamate muscle relaxants described in Table VIII were successfully characterized by the preparation of acetyl derivatives.

Table VIII. Acetyl Derivatives

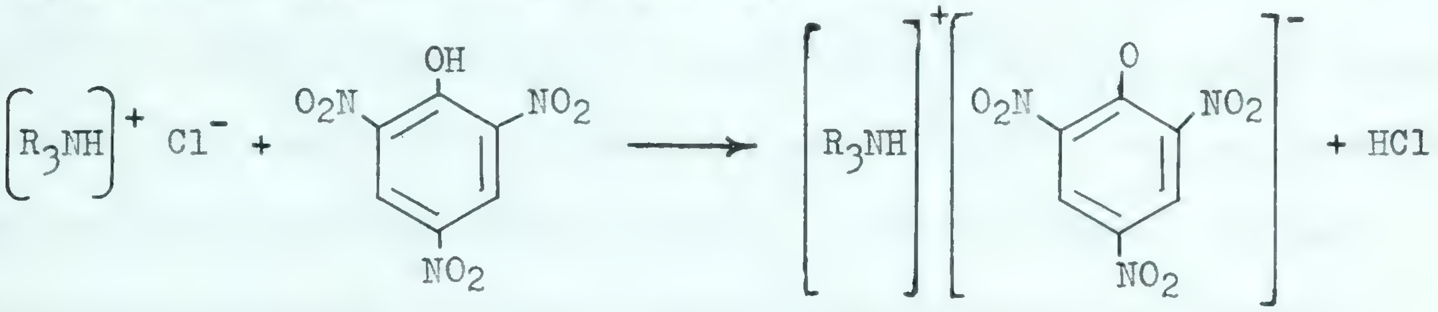
Parent Compound	Melting Point, °C		Type of Derivative (parent compound:reagent)
	Found (corr.)	Literature	
Methocarbamol	84.0-85.0		1:2
Styramate	96.5-98.0		1:2
Carisoprodal	87.5-88.5		1:1
Meprobamate	127.5-128.5	125.0-130.0 (41,42)	1:2

Styramate and methocarbamol both contain a secondary hydroxyl group as well as a primary unsubstituted carbamate group. The elemental analyses of the derivatives indicated that the parent compounds had been attacked by two acetyl groups. In an effort to substantiate this, the integrated nuclear magnetic resonance spectra were determined for these two derivatives, and the integration curves revealed the presence of nineteen protons for the methocarbamol derivative and fifteen protons for the

styramate derivative. This information complimented the elemental analyses data, by confirming that the investigation had yielded the 2:1 products. Thus in the case of methocarbamol and styramate, the acetyl group has attacked both the secondary hydroxyl and the primary carbamate nitrogen.

8) Picrates

Picrates have long been recognized as a satisfactory criterion for the qualitative identification of organic bases, and their formation may be expressed by the following general equation:



A review of the literature has revealed that relatively few picrates have been reported for the basic amino muscle relaxants. Delaby et al. (92) have reported the preparation of the picrate of phenyramidol with a melting point of 151.0-152.0 C. Picrates prepared in this investigation, together with literature values are tabulated in Table IX.

Table IX. Picrate Derivatives

Parent Compound	Melting Point, °C		Molecular Weight	
	Found (corr.)	Literature	Calculated	Found
Cycrimine	145.0-146.5		516.50	512.99
Procyclidine	63.5-65.0		516.50	514.02
Phenyramidol	153.5-154.5	151.0-152.0 (92)	443.38	442.89

Trihexyphenidyl and biperiden picrate were not obtained in a crystalline state. These products initially formed an oily mass, and repeated crystallization attempts from various solvents (ethanol,methanol,acetone),

proved unsuccessful.

The picrate derivatives were obtained in good yield, were easily purified, and had sharp, well distributed, characteristic melting points.

9) Reineckates

Numerous organic bases having both pharmaceutical application (122, 123,124) and non-pharmaceutical application (125) have been characterized with ammonium reineckate. The general equation for the reaction of amine salts in aqueous solution with ammonium reineckate is illustrated as follows:

$$\left[R_3NH\right]^+Cl^- + NH_4^+\left[Cr(NH_3)_2(SCN)_4\right]^- \longrightarrow \left[R_3NH\right]^+\left[Cr(NH_3)_2(SCN)_4\right]^- + NH_4Cl$$

Under the weakly acidic conditions used (pH 5-6), near quantitative yields of the reineckate were obtained. In every instance, the amine muscle relaxants studied formed the anhydrous derivative. This was verified by elemental analyses (carbon, hydrogen, nitrogen) and the gravimetric determination of chromium calculated as Cr_2O_3 (Table X).

Table X. Reineckate Derivatives

Parent Compound	Melting Point °C (corr.)	Combustion Assay Data		
		Recovery %Cr	Molecular Weight	
			Calculated	Found
Biperiden	160.0-164.0 (D)	99.03	630.88	624.76
Cycrimine	154.0-158.0 (D)	99.29	606.86	602.55
Procyclidine	149.0-154.0 (D)	101.28	606.86	614.63
Trihexyphenidyl	159.0-162.0 (D)	98.93	620.89	614.25
Phenyramidol	156.0-158.0 (D)	99.28	533.69	529.85

Abbreviations: D = decomposition.

Several workers (122,125) have reported that excessive heat will decompose reineckates, and for this reason these derivatives were prepared and recrystallized at room temperature.

All reineckates prepared in this problem were observed to have a reasonably sharp decomposition range. However, some overlap in the melting ranges of the derivatives was evident, thus necessitating the preparation of other derivatives for conclusive identification.

10) Tetraphenylborates

The instantaneous reaction of sodium tetraphenylboron with basic organic nitrogen amines proceeds in accordance with the equation (111):

$$\left[R_3NH\right]^+Cl^- + Na^+\left[B(C_6H_5)_4\right]^- \longrightarrow \left[R_3NH\right]^+\left[B(C_6H_5)_4\right]^- + NaCl$$

Precipitation of amine TPB salts depends upon the presence of protonated amines(126). As strong acid solutions will decompose the TPB ion rapidly (128), buffers of pH 4 to 6 are in general the most satisfactory for the protonation and precipitation of these derivatives. Since aqueous solutions of the amine hydrochloride salts were used, initial protonation was not a factor to be considered.

Analytical quantitative procedures were not employed for the preparation of these derivatives and therefore quantitative yields of the product were not obtained, however highly acceptable yields were recorded (88 to 94%).

It has been stated that washing TPB salts free of excess reagent, and drying under vacuum yields compounds which are sufficiently pure for characterization purposes (127,129). In this investigation, the TPB derivatives were recrystallized to obtain well defined crystalline products for infrared spectra. Crane (129) has shown several TPB salts to be heat labile, and for this reason the derivatives were prepared and recrystallized at room temperature.

Since good yields of easily purifiable product were obtained and well distributed melting points were noted, the tetraphenylborate salts proved to be highly desirable derivatives for qualitative identification.

When titrated in nonaqueous media, excellent quantitative recoveries were obtained for all derivatives. These titration values were utilized to determine the molecular or equivalent weights which are reported in Table XI, together with the melting point data.

Table XI. Tetraphenylborate Derivatives

Parent Compound	Melting Point °C (corr.)	Molecular Weight	
		Calculated	Found
Biperiden	208.0-209.5	631.67	629.90
Cycrimine	157.5-159.0	607.64	604.50
Procyclidine	148.0-150.0	607.64	603.20
Trihexyphenidyl	157.0-159.0	621.69	625.98
Phenyramidol	124.0-126.0	534.53	536.24

11) Methiodides

Tertiary amines can be characterized by the formation of quaternary salt type derivatives according to the following general equation.

$$R_3N: + CH_3I \longrightarrow \left[R_3NCH_3\right]^+ I^-$$

The methiodides of several of the amino muscle relaxants included in this study have been reported, and are listed in comparison with the observed melting points in Table XII.

Table XII. Methiodide Derivatives

Parent Compound	Melting Point, °C	
	Found (corr.)	Literature
Biperiden	207.0-208.0	
Cycrimine	145.0-146.0	235.0-237.0 (131)
Procyclidine	204.0-205.0 (D)	204.0-205.0 (D)(29,130)
Trihexyphenidyl	208.0-208.5	203.0-204.0 (29.130)
Phenyramidol	165.5-166.5	164.0-166.0 (34)

Abbreviations: D = decomposition.

As is immediately apparent from the foregoing data, the observed melting point of cycrimine methiodide is radically different from that reported in the literature. No apparent reason for this anomaly could be found except that reference (131) did not include the elemental analysis of their derivative.

The methiodides were readily prepared and easily purified derivatives with sharp melting points. Their only apparent disadvantage is the overlap in the melting ranges which precludes their use for qualitative identification without the preparation of additional derivatives.

12) <u>Chloroplatinates</u>

Chloroplatinic acid can form crystalline addition compounds with amines and is useful for the identification of tertiary bases which undergo only a small number of reactions. Stoichiometric relationships involved in the preparation of the chloroplatinates depend upon whether the drug is mono- or dibasic.

Monobasic:

$$2\left[R_3NH\right]^{+}Cl^{-} + H_2\left[PtCl_6\right] \longrightarrow \left[R_3NH\right]_2^{++}\left[PtCl_6\right]^{=} + 2HCl$$

Dibasic:

$$\left[HNR_3{-}R_3NH\right]^{++} 2Cl^{-} + H_2\left[PtCl_6\right] \longrightarrow \left[HNR_3{-}R_3NH\right]^{++}\left[PtCl_6\right]^{=} + 2HCl$$

All of the amino muscle relaxants studied in this investigation were monobasic.

The literature reveals no record that chloroplatinic acid has been used to characterize any of the amino muscle relaxants in this problem. All chloroplatinate derivatives decomposed on heating but the decomposition ranges are reproducible and characteristic. Recrystallization was a problem encountered during the preparation of these derivatives. Generally the chloroplatinates were only sparingly soluble in ethanol (95%) or methanol, and any amount of heating resulted in a partial or complete decomposition of the product. In order to avoid this the

chloroplatinates were purified by washing well with cold distilled water prior to submission for elemental analyses. Excellent results were obtained and decomposition ranges were characteristic in each instance with no overlap.

The chloroplatinates prepared during this investigation are presented in Table XIII.

Table XIII. Chloroplatinate Derivatives

Parent Compound	Melting Point °C (corr.)	Type of Derivative (base:reagent)
Biperiden	142.0-147.0 (D)	2:1
Cycrimine	179.0-182.0 (D)	2:1
Procyclidine	199.0-203.0 (D)	2:1
Trihexyphenidyl	132.0-137.0 (D)	2:1
Phenyramidol	156.0-160.0 (D)	2:1

Abbreviations: D = decomposition.

13) Parent Compounds

The individual specific melting point of each parent skeletal muscle relaxant is an important part of the identification scheme, and all are included here (Table XIV) in order to compliment the previous work on derivatives.

Table XIV. Melting Points of the Muscle Relaxants

Parent Compound	Melting Point, °C	
	As received (corr.)	Literature
Mephenesin	70.5-71.5	70.0-72.0 (132)
DEP	61.5-62.5	61.0-61.6 (45)
Meprobamate	104.0-105.5	105.0-106.0 (43)
Methocarbamol	91.5-93.0	92.0-94.0 (46)

Table XIV. - Continued

Parent Compound	Melting Point, °C As received (corr.)	Melting Point, °C Literature
Styramate	110.5-112.0	111.0-112.0 (133)
Carisoprodal	92.0-93.0	89.0-91.0 (134)
Phenyramidol HCl	141.0-143.0	140.0-142.0 (34)
Biperiden HCl	268.0-270.0 (D)	...
Cycrimine HCl	234.0-236.0 (D)	236.0-237.0 (D)(131) 233.0-233.5 (D)(27)
Procyclidine	225.5-226.5 (D)	227.0 (D)(130) 226.0-227.0 (D)(29)
Trihexyphenidyl	255.5-257.5 (D)	241.0-243.0 (D)(130) 255.0 (D)(29) 258.5 (D)(23)

Abbreviations: D = decomposition.

In summary, the prepared derivatives for the characterization of the alcoholic and carbamate moieties are indicated by a plus sign in the following table (Table XV). Derivatives of the amine moiety are briefly outlined in a comparable table (Table XVI).

B. INFRARED SPECTROSCOPY.

Stimson and O'Donnell (135) in 1952, were among the first to introduce the potassium bromide disk technique to infrared analysis. Since then it has enjoyed extensive usage in the fields of both quantitative and qualitative analysis. Its application to the qualitative identification of drugs is exemplified by its inclusion in pharmaceutical compendia (39,41) and literature (97,136).

It was believed that the infrared spectra would prove of considerable value for the interpretation of structural features and aid in establishing the identity of these compounds.

Table XV. Derivatives of the Alcohol and Carbamate Moieties

Parent Compound	Alcoholic				Carbamate		
	Phenyl or 1-Naphthyl Carbamates	3,5-Dinitrobenzoates	Acid 3-Nitrophthalates	2,4-Dinitrobenzenesulfenates	Xanthyl Derivatives	Diphenylmethyl Derivatives	Acetyl Derivatives
Mephenesin	+	+		+			
DEP	+	+	+				
Methocarbamol	+	+	+	+	+		+
Styramate	+		+	+	+		+
Phenyramidol	+		+	+			
Meprobamate					+	+	+
Carisoprodal					+	+	+

Table XVI. Derivatives of the Amine Moiety

Parent Compound	Picrates	Reineckates	Tetraphenylboarates	Methiodides	Chloroplatinates
Biperiden		+	+	+	+
Cycrimine	+	+	+	+	+
Procyclidine	+	+	+	+	+
Trihexyphenidyl		+	+	+	+
Phenyramidol	+	+	+	+	+

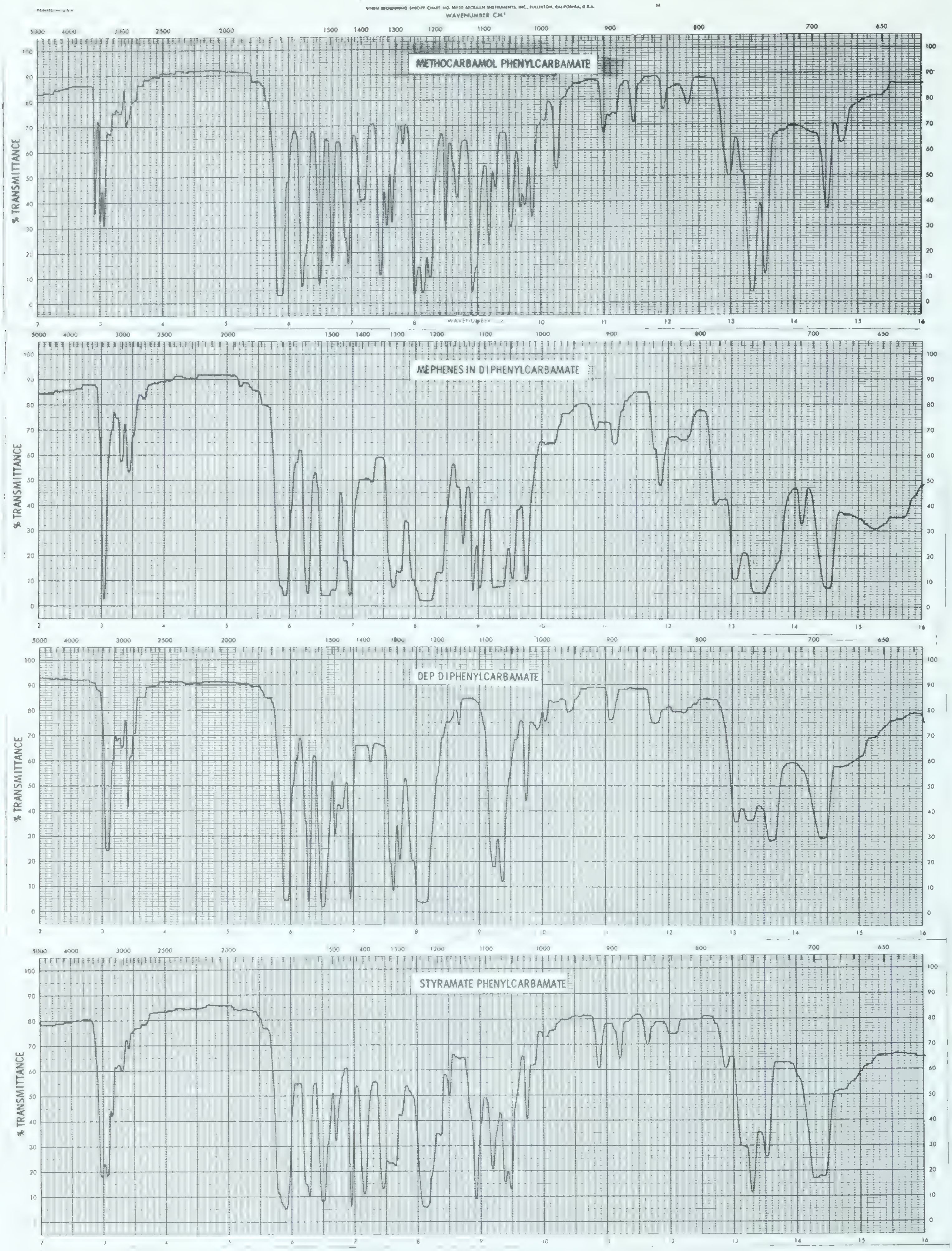


Figure 1. Infrared spectra of the phenyl and 1-naphthyl carbamates.

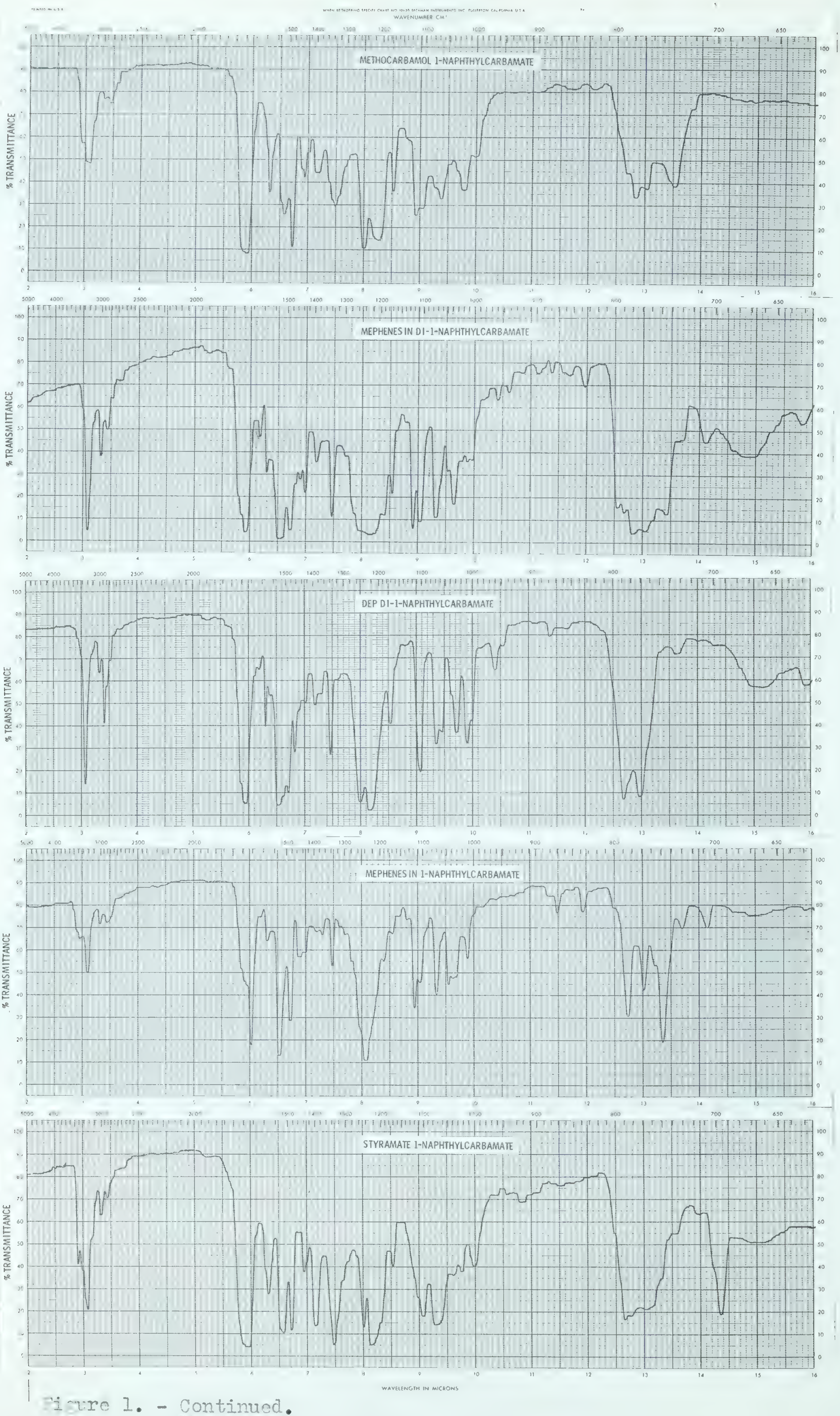


Figure 1. - Continued.

[illegible]

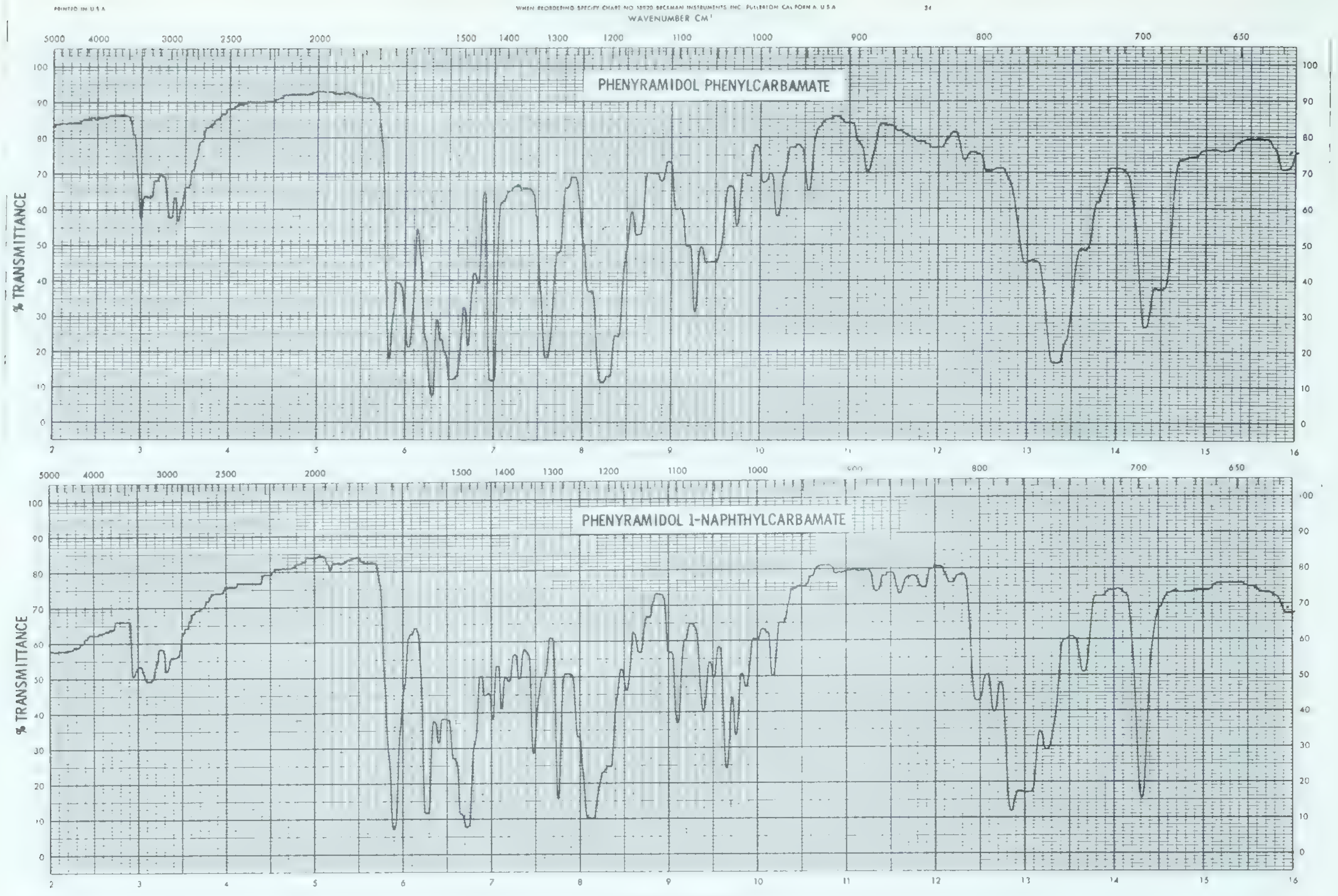


Figure 1. - Continued.

All spectra* were completely reproducible when prepared according to the potassium bromide procedure previously outlined (p. 39). A clear blank potassium bromide pellet was scanned in the 2-16 micron region and showed negligible absorption in the 3500 and 1650 cm.$^{-1}$ regions (OH stretching and H-O-H bending vibrations). However, a potassium bromide disk was placed in the path of the reference beam to compensate for possible traces of absorbed moisture from the atmosphere.

For each spectrum presented, the shaking and pressing times will be included.

1) Phenyl and 1-Naphthyl Carbamates

The concentration of the derivatives in the potassium bromide pellets was usually 0.5%. Mephenesin diphenylcarbamate and mephenesin di-1-naphthylcarbamate were determined at a concentration of 1.0%. All samples were shaken for fifteen seconds in the amalgamator and pressed for three minutes.

These spectra (Figure 1) exhibit numerous commmon bands, in keeping with the carbamate grouping present. Weak to medium absorption bands throughout the 3400-3200 cm.$^{-1}$ region due to NH stretching vibrations and weak absorption throughout the 3050-2850 cm.$^{-1}$ aromatic and aliphatic CH stretching region is generally common to all spectra. A strong amide I band (carbonyl absorption of the carbamate moiety)

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* The interpretation of all spectra was based upon:

Nakanishi, K., "Infrared Absorption Spectroscopy," 2nd Edition, Holden-Day, Inc., San Francisco, 1964.

L.J. Bellamy, "The Infra-red Spectra of Complex Molecules," 2nd Edition, John Wiley & Sons, Inc., New York, 1959.

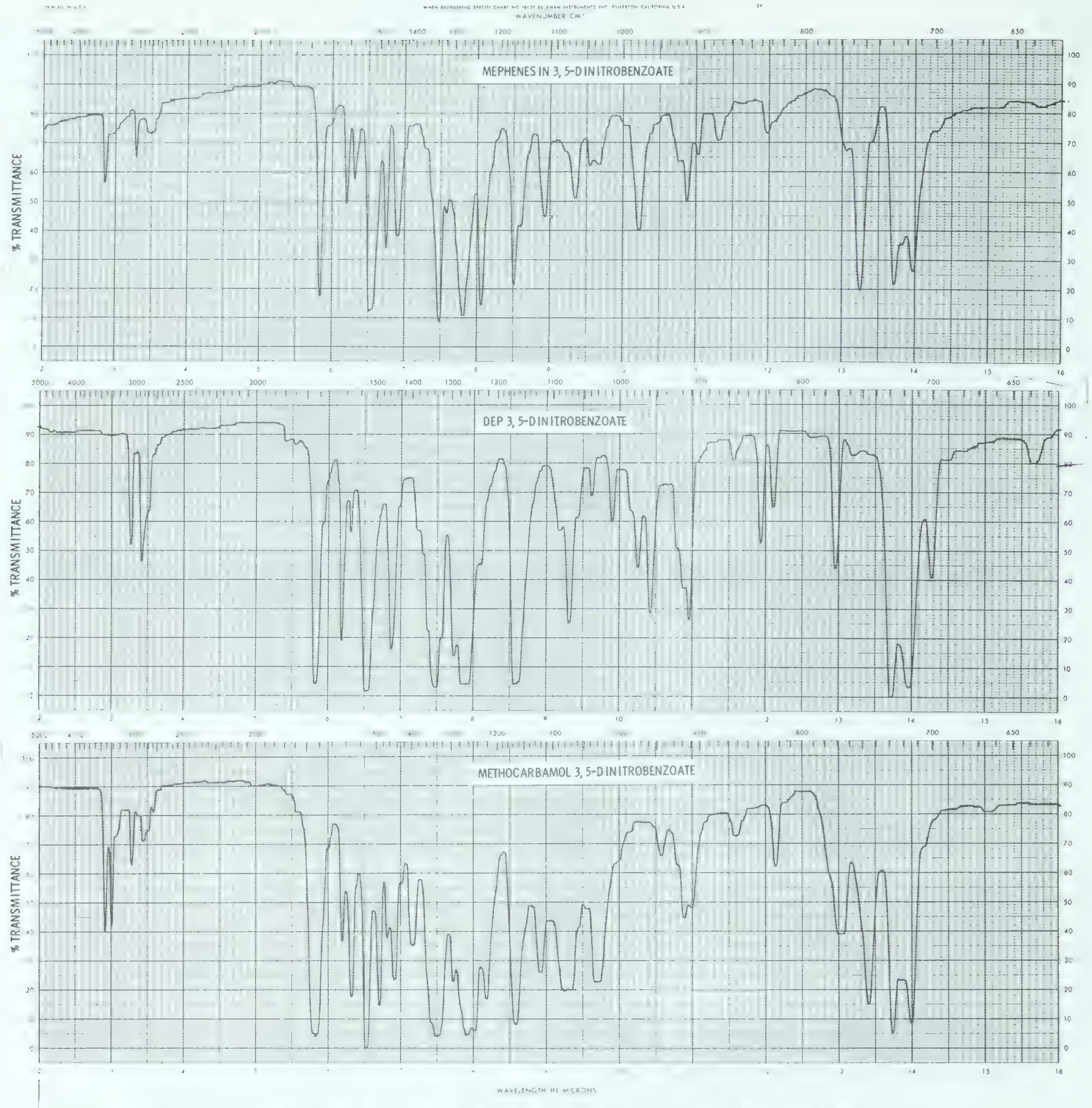


Figure 2. Infrared spectra of the 3,5-dinitrobenzoates.

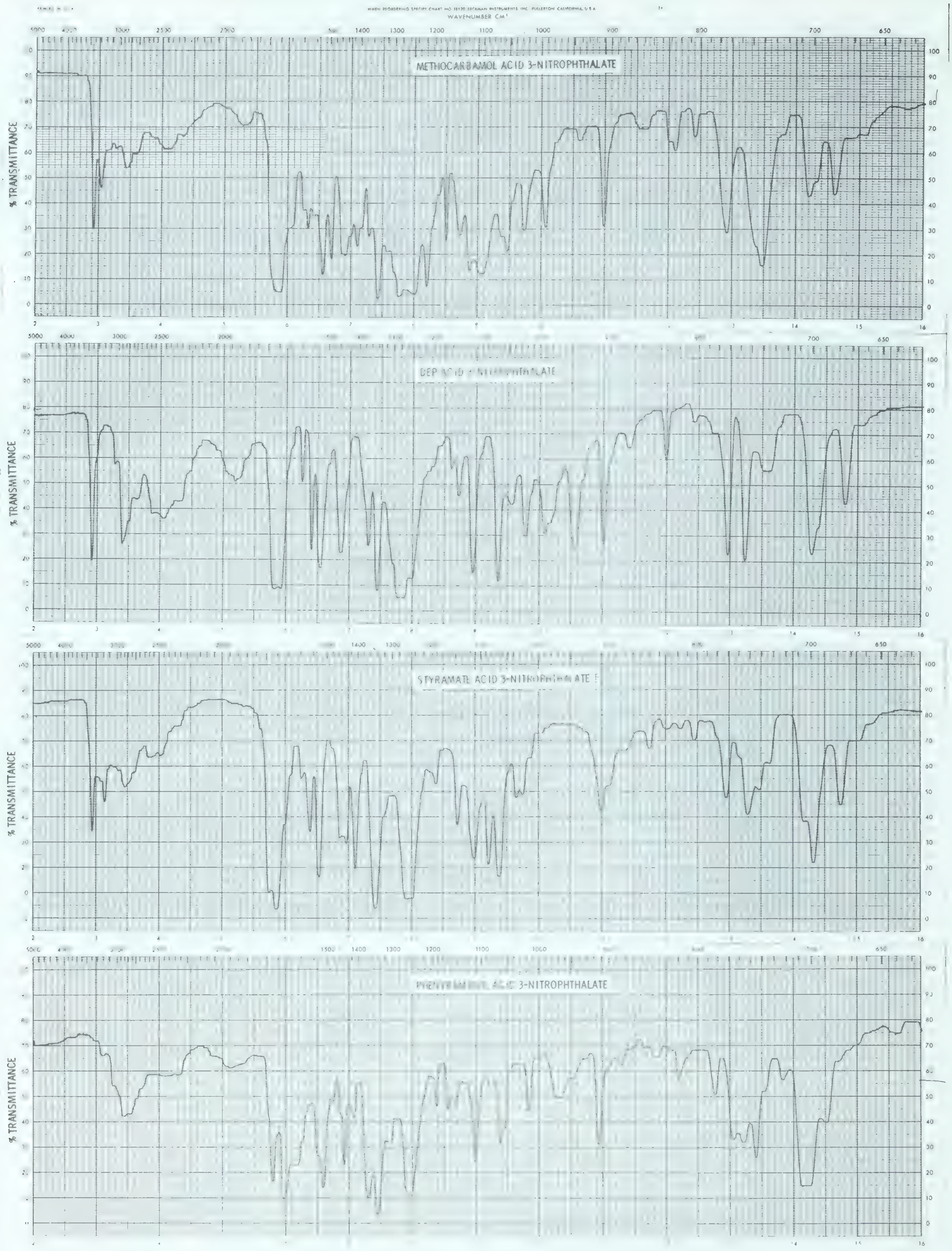


Figure 3. Infrared spectra of the acid 3-nitrophthalates.

was present in the 1735-1690 cm.$^{-1}$ region, and strong C=C skeletal in-plane aromatic ring vibrations in the 1600-1500 cm.$^{-1}$ region are obvious. The 8-16 micron region is very characteristic and is most useful for differentiating these compounds.

2) 3,5-Dinitrobenzoates

Very characteristic spectra of the benzoates could be obtained (Figure 2) by using pellet concentrations of 0.75%. Samples were shaken for fifteen seconds and pressed for three minutes.

Mephenesin 3,5-dinitrobenzoate shows a weak to medium band at 3500-3400 cm.$^{-1}$ due to the OH stretching of the hydroxyl group present. Methocarbamol 3,5-dinitrobenzoate exhibits two modes at 3500 and 3400 cm.$^{-1}$ due to asymmetric and symmetric NH stretching vibrations of the primary carbamate grouping. Weak to medium absorption throughout the 3100-2850 cm.$^{-1}$ region due to aromatic and aliphatic CH stretching are common to all spectra. Ester carbonyl absorption is indicated by a strong band at 1725 cm.$^{-1}$. Strong bands at 1540 and 1350 cm.$^{-1}$ represents the nitro asymmetrical and symmetrical stretchings respectively. Strong doublet modes at 730 and 710 cm.$^{-1}$ are characteristic of the 3,5-dinitrobenzoates in this investigation.

3) Acid 3-Nitrophthalates

The pellet concentrations of the acid 3-nitrophthalates varied as follows: phenyramidol and styramate 0.4%, methocarbamol and DEP 0.75%. All were shaken for fifteen seconds and pressed for three minutes.

The spectra (Figure 3) exhibit many complimentary bands as exemplified by the absorption modes at 1735 and 1700 cm.$^{-1}$ due to the carbonyl moieties found in the ester and carboxylic acid groups. DEP acid 3-nitrophthalate shows a strong absorption at 3450 cm.$^{-1}$ due to the primary hydroxyl group present. Methocarbamol and styramate

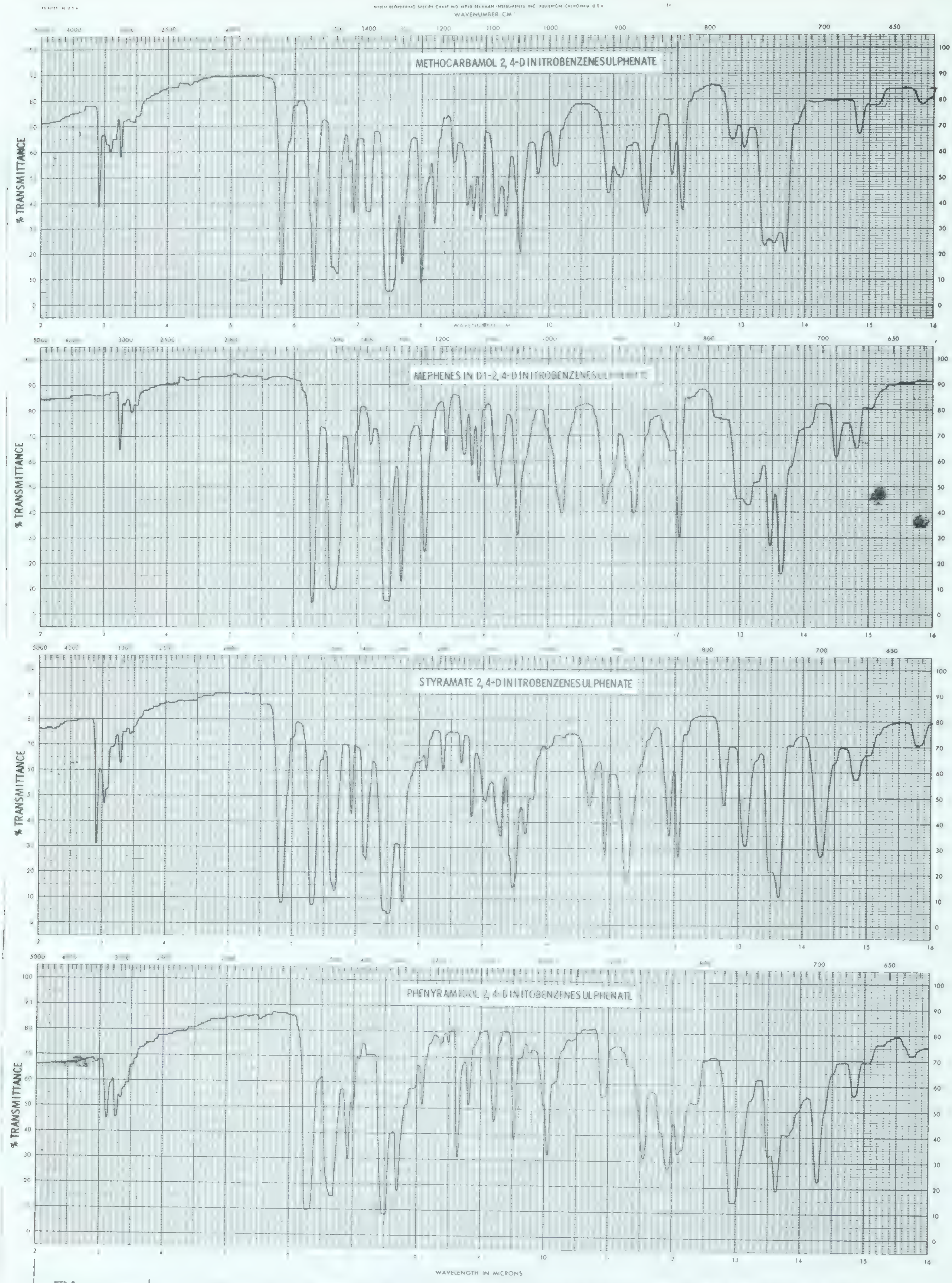
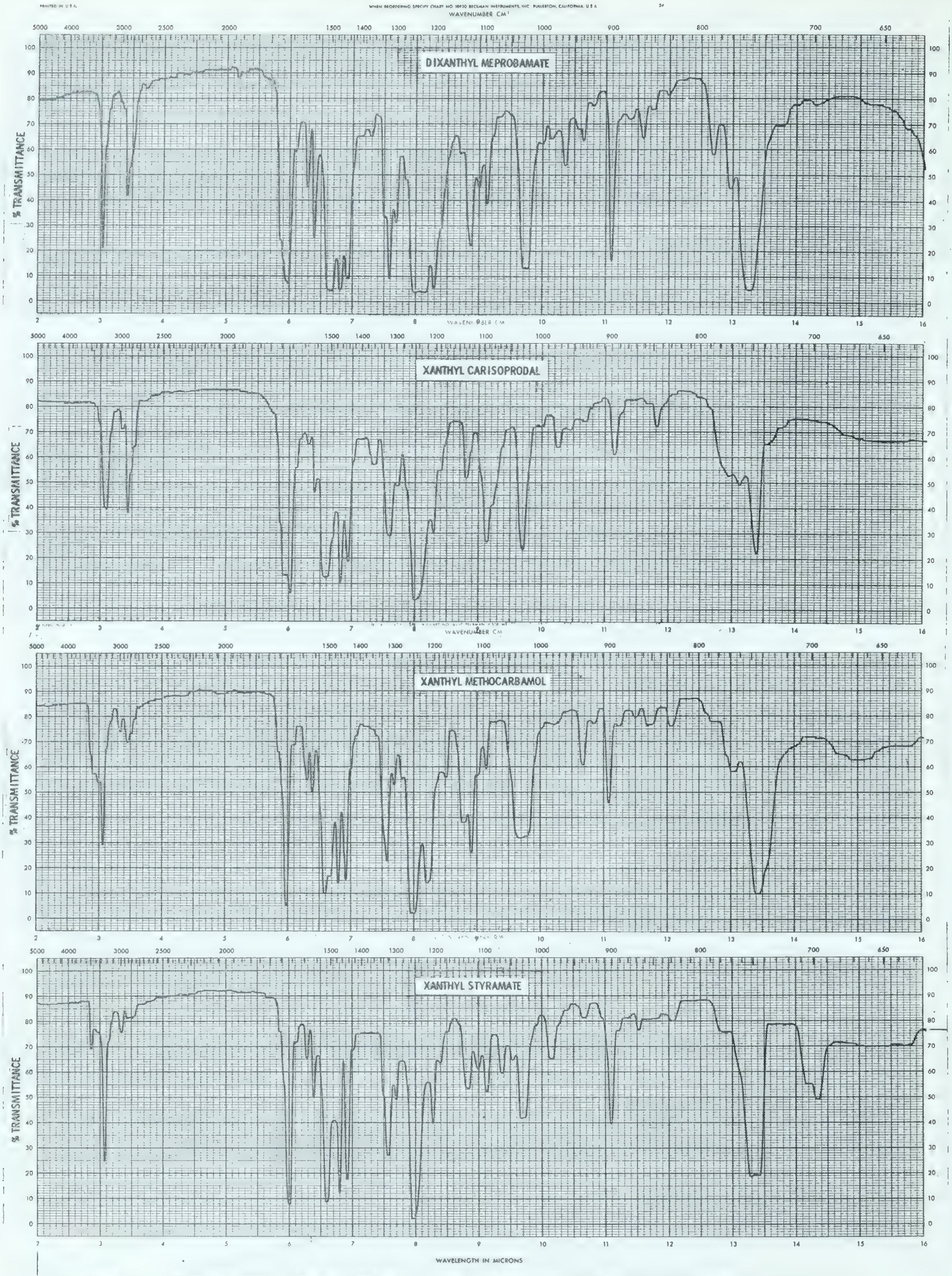


Figure 4. Infrared spectra of the 2,4-dinitrobenzenesulphenates.


DIXANTHYL MEPROBAMATE

XANTHYL CARISOPRODAL

XANTHYL METHOCARBAMOL

XANTHYL STYRAMATE

Figure 5. Infrared spectra of the xanthyl derivatives.

acid 3-nitrophthalate both exhibit two free NH stretching modes near 3400 and 3250 cm.$^{-1}$ corresponding to the asymmetric and symmetric motions of the hydrogen atom in the carbamate moiety. The OH stretching frequency of the acid groups occur as broad bands with a series of minor peaks over the 3000-2500 cm.$^{-1}$ region. The O-H out-of-plane bending of an acid dimer is indicated by a relatively broad band of medium intensity at 920 cm.$^{-1}$.

4) 2,4-Dinitrobenzenesulfenates

Pellet concentrations of 0.5% were employed for all derivatives, with the exception of phenyramidol 2,4-dinitrobenzenesulfenate which was determined at a concentration of 0.8%. All samples were shaken for fifteen seconds in the amalgamator and subjected to five minutes pressure.

Methocarbamol and styramate 2,4-dinitrobenzenesulfenate esters both show characteristic bands originating in primary carbamate NH stretching modes (3420 cm.$^{-1}$ and 3300 cm.$^{-1}$). In addition, the carbonyl absorption of the carbamate results in an amide I band at 1725 cm.$^{-1}$ for these derivatives (Figure 4). Neither phenyramidol nor mephenesin 2,4-dinitrobenzenesulfenates demonstrate the aforementioned absorptions.

All spectra show an intense band at 1340 cm.$^{-1}$ representing the nitro symmetrical stretching vibrations. The presence of an aromatic group is illustrated by the 3040 cm.$^{-1}$ and 1590 cm.$^{-1}$ aromatic bands (=CH and C=C).

5) Xanthyl Derivatives

The pellet concentration of dixanthyl meprobamate was 0.7%, while the other derivatives had a concentration of 0.4%. Samples were shaken for fifteen seconds and then were subjected to three minutes pressure.

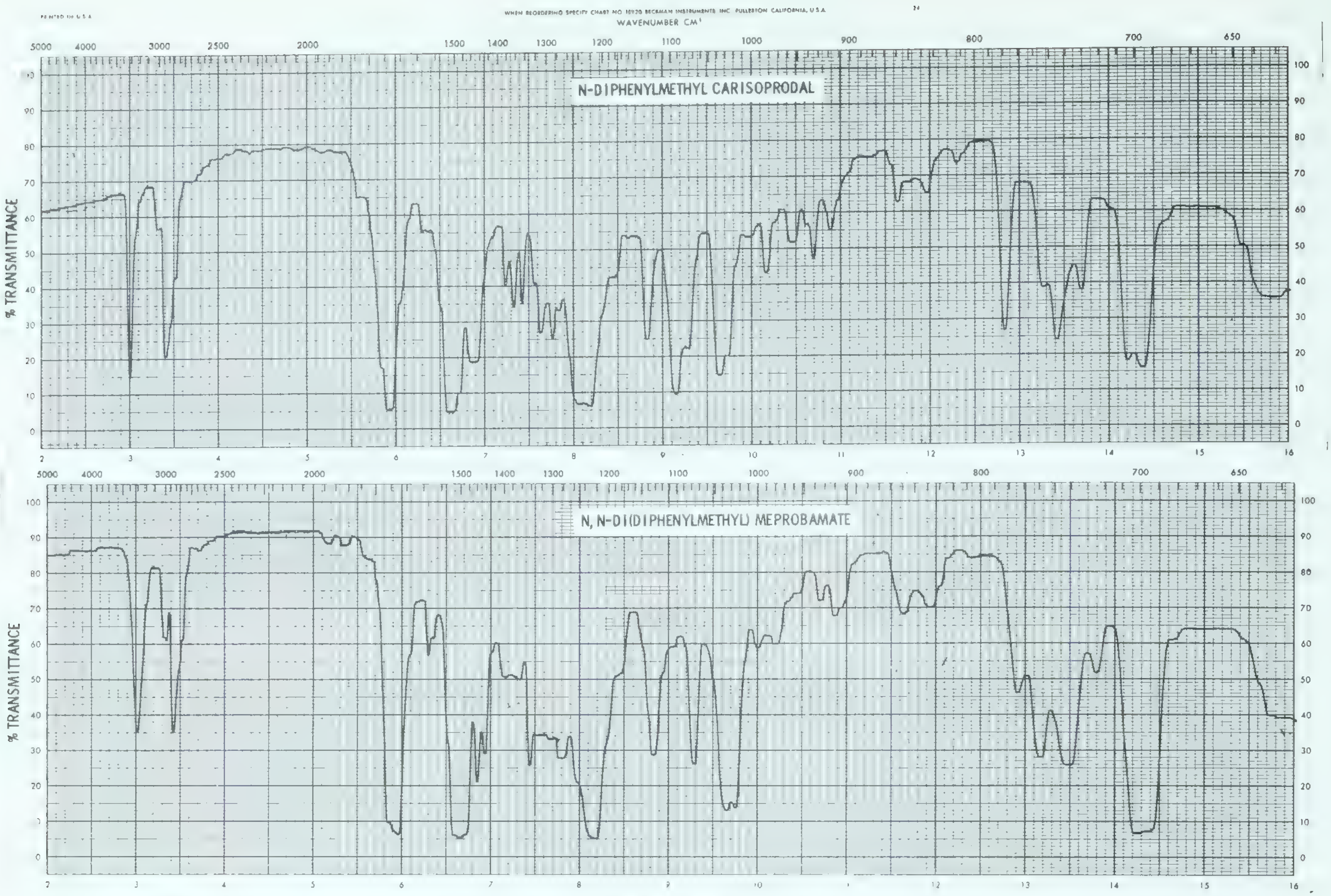


Figure 6. Infrared spectra of the diphenylmethyl derivatives.

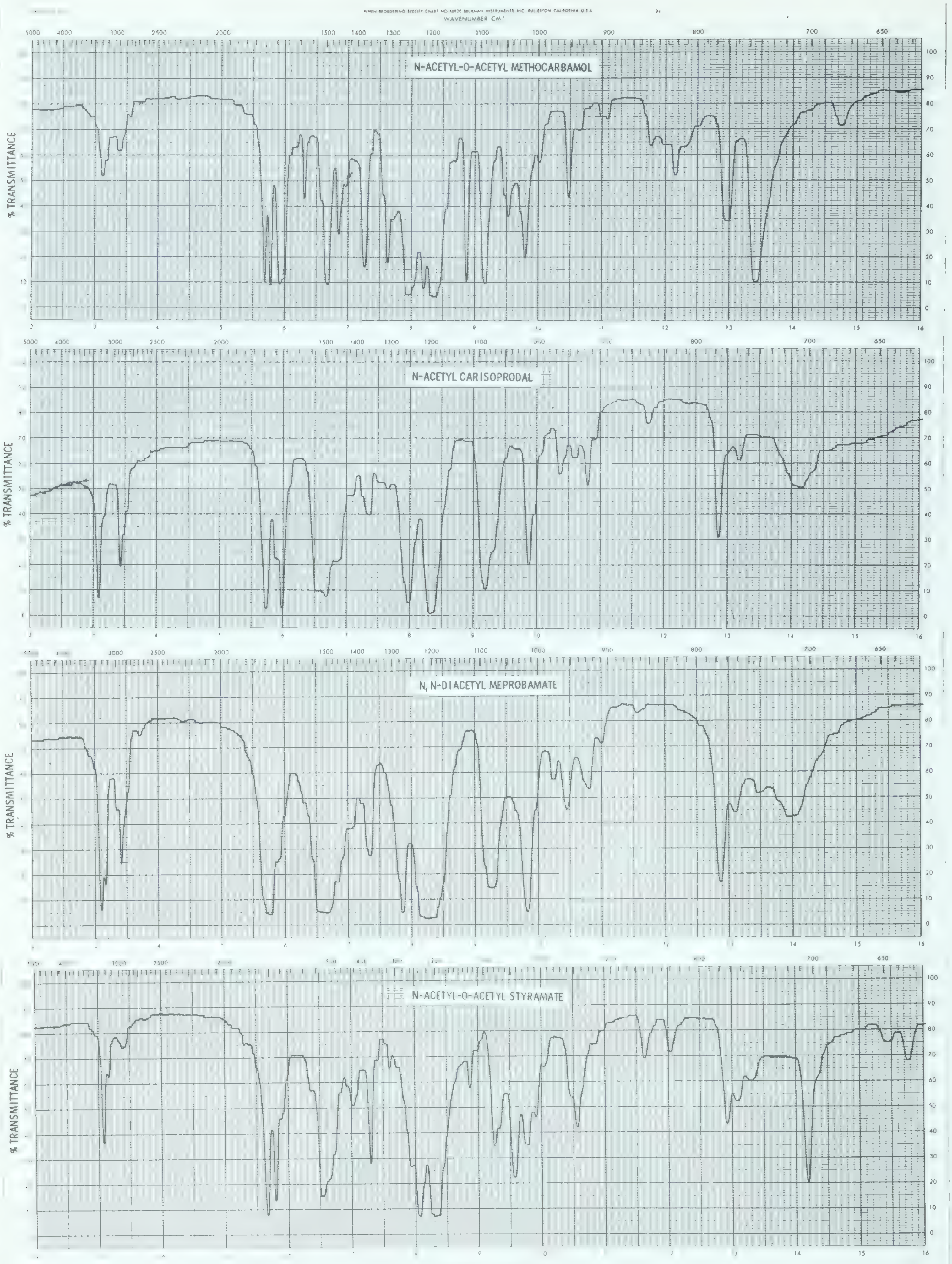


Figure 7. Infrared spectra of the acetyl derivatives.

All spectra revealed a single absorption at 3300 cm.$^{-1}$ due to the secondary carbamate NH stretching vibration. Xanthyl methocarbamol and xanthyl styramate showed OH stretching at 3500 cm.$^{-1}$ and 3400 cm.$^{-1}$ respectively. Medium bands at 2950 cm.$^{-1}$ for the carisoprodal and meprobamate derivatives indicated alkane CH stretching frequencies. A strong amide I band (carbonyl absorption) at 1780-1760 cm.$^{-1}$ was obvious in all spectra (Figure 5).

The xanthyl part of the derivatives contained an aryl ether and this was exemplified by the presence of a strong band at 1250 cm.$^{-1}$ due to the =C-O stretching vibration for all derivatives. A strong broad band is evident in the "fingerprint" region at 750 cm.$^{-1}$ for all xanthyl derivatives studied.

6) Diphenylmethyl Derivatives

A pellet concentration of 1.0% was used for the determination of the infrared spectra of these derivatives. Both samples were shaken for fifteen seconds in the amalgamator and then were pressed for five minutes.

Upon inspection of Figure 6, medium bands in the 3400-3300 cm.$^{-1}$ range are characteristic for these compounds due to NH stretching vibrations. Medium absorption throughout the 3050-2850 cm.$^{-1}$ aromatic and aliphatic CH stretching region is common to both spectra. Strong carbonyl stretching *ca.* 1700 cm.$^{-1}$ from the carbamate linkage is obvious, along with strong C=C skeletal in-plane phenyl ring vibrations *ca.* 1600 cm.$^{-1}$ and 1500 cm.$^{-1}$. The 8-16 micron region is very characteristic and is most useful for differentiating these compounds.

7) Acetyl Derivatives

A pellet concentration of 0.4% for the complete series of acetyl derivatives resulted in spectra showing good resolution. All samples

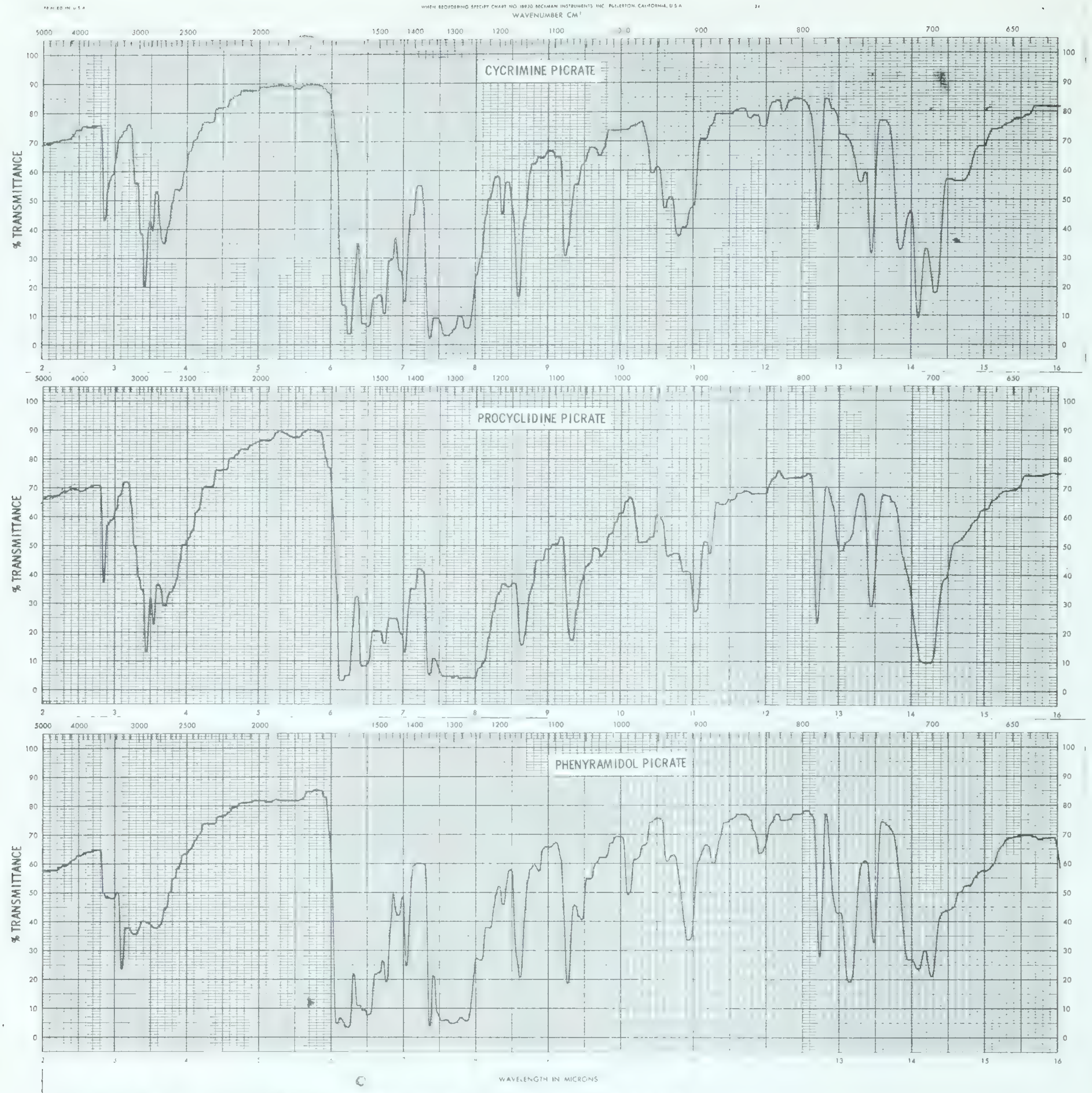


Figure 8. Infrared spectra of the picrates.

were shaken for fifteen seconds and pressed for three minutes.

A weak to medium NH stretching absorption at 3300-3200 cm.$^{-1}$ and a weak band throughout the 3000-2900 cm.$^{-1}$ aliphatic CH stretching region is common to all spectra.

The carbonyl absorption of each particular compound is extremely charateristic as is evident from Figure 7. The methocarbamol derivative appears to have three carbonyl absorptions (1750, 1725 and 1695 cm.$^{-1}$); styramate and carisoprodal derivatives exhibit two carbonyl absorptions at 1750-1725 cm.$^{-1}$ and 1740-1680 cm.$^{-1}$ respectively; and the meprobamate derivative appears to have only a single broad carbonyl absorption at 1730 cm.$^{-1}$.

The acetate ester found in both styramate and methocarbamol shows a typical C-O stretch at 1235 cm.$^{-1}$ and 1250 cm.$^{-1}$ respectively.

Infrared spectra of meprobamate and carisoprodal acetyl derivatives are of interest since they are aliphatic and contain no absorptions charateristic of aromatic or alkene compounds.

8) Picrates

The pellet concentrations of the picrates which resulted in spectra showing good resolution varied as follows: phenyramidol 0.4%, cycrimine 0.75%, procyclidine 1.0%. The samples were all shaken for fifteen seconds and pressed for five minutes.

Medium to weak absorption in the 3500 cm.$^{-1}$ region (OH stretching vibration) and medium absorption throughout the 3050-2850 cm.$^{-1}$ aromatic and aliphatic CH stretching region is common to all spectra (Figure 8). Multiple or broad bands in the 2700-2250 cm.$^{-1}$ region due to NH^+ stretching vibrations, overtones, and combinations are also present. Strong C=C skeletal in-plane phenyl ring vibrations ca. 1600 cm.$^{-1}$ and 1500 cm.$^{-1}$ are noted. Intense bands at 1560 cm.$^{-1}$ and 1360 cm.$^{-1}$

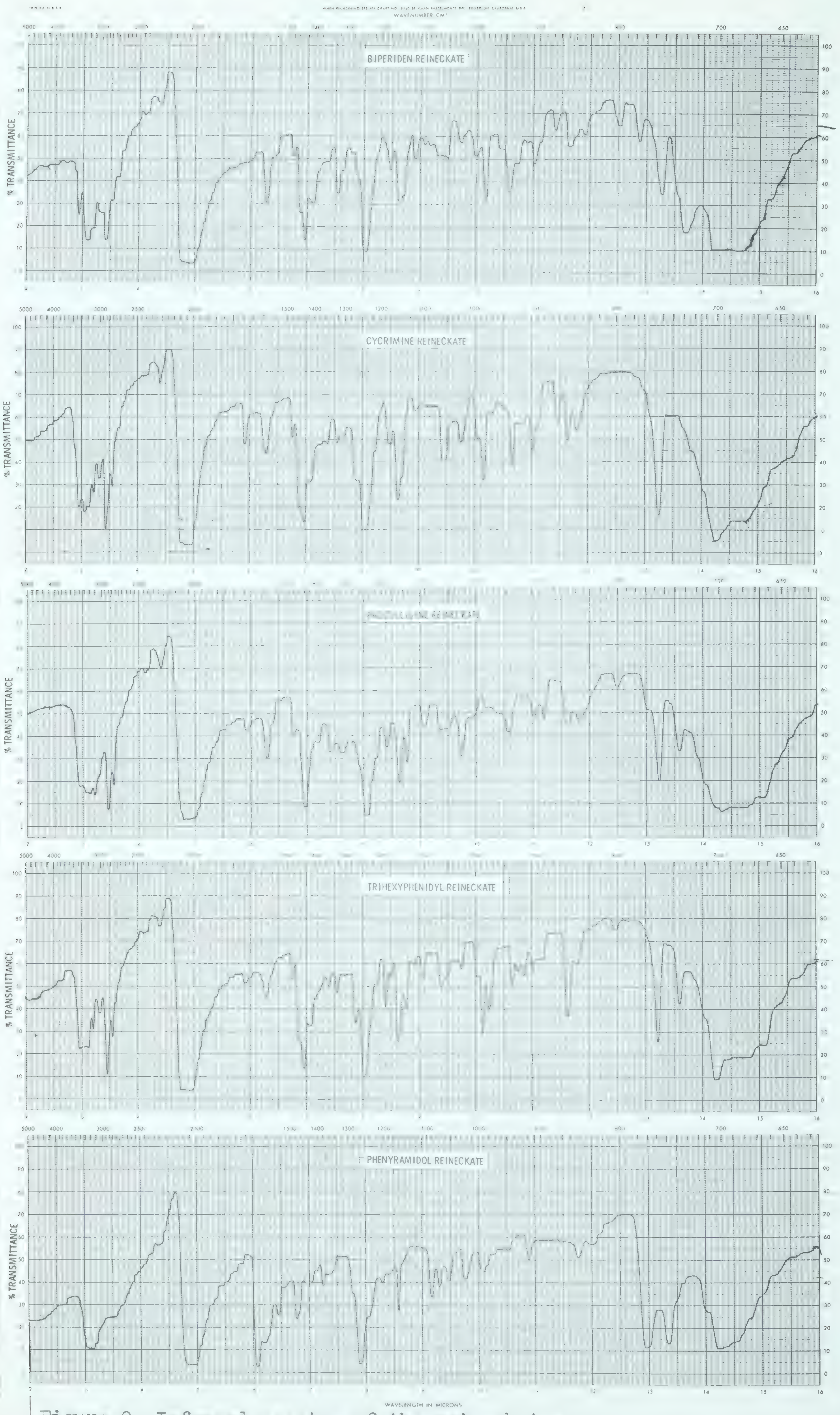


Figure 9. Infrared spectra of the reineckates.

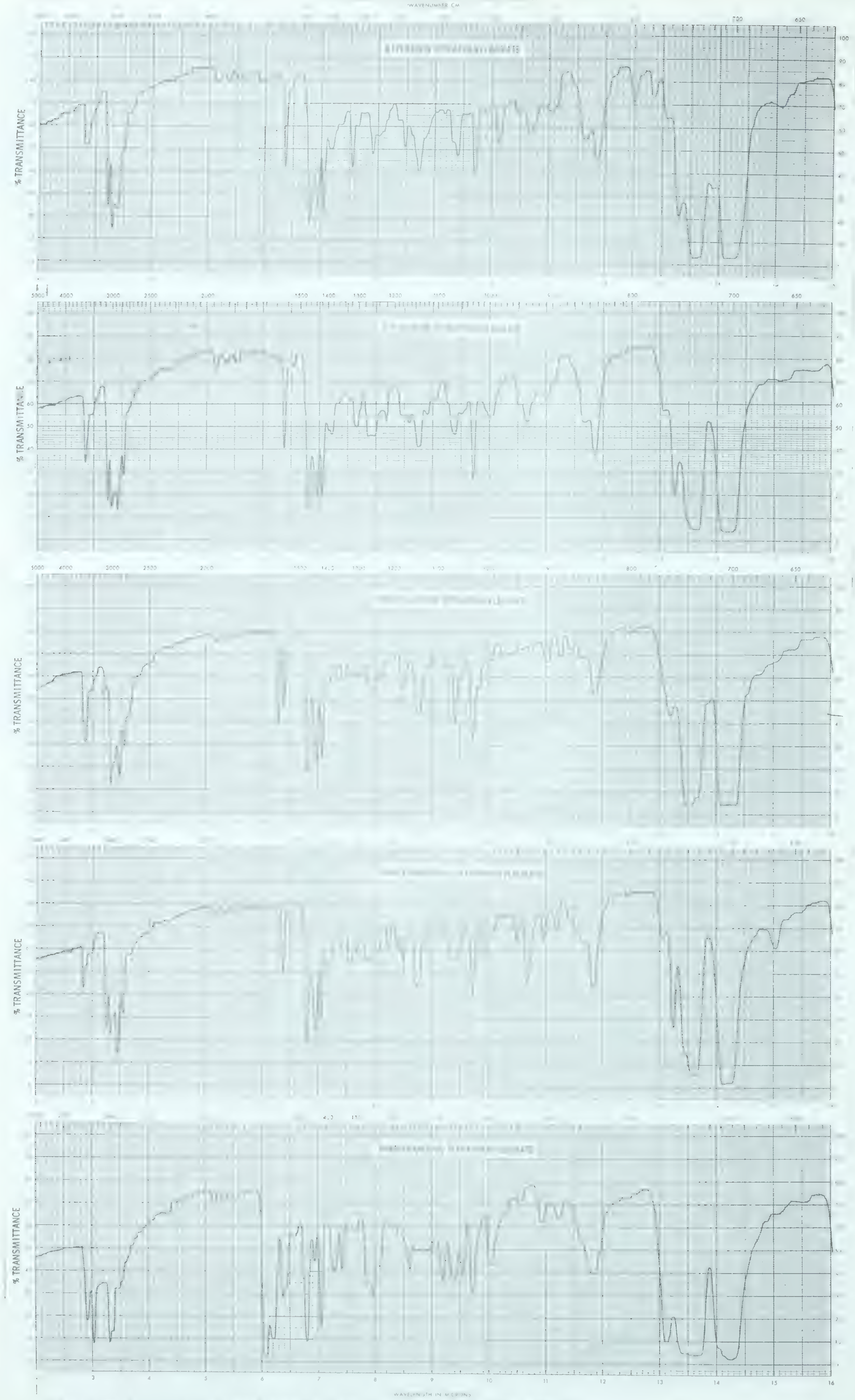


Figure 10. Infrared spectra of the tetraphenylborates.

represent the nitro asymmetrical and symmetrical stretching vibrations respectively.

In the "fingerprint" region two sharp characteristic bands, common to the three spectra, appear at 785 cm.$^{-1}$ and 740 cm.$^{-1}$.

9) Reineckates

Positive differentiation of the reineckates was obtained with the following pellet concentrations: cycrimine, phenyramidol and trihexyphenidyl 1.1%, biperiden 1.4%, procyclidine 1.8%. All samples were shaken for fifteen seconds and pressed for five minutes with the exception of the cycrimine and phenyramidol derivatives which were subjected to eight minutes pressure.

The spectra (Figure 9) of the reineckate derivatives were generally less detailed than the corresponding spectra of the parent hydrochloride salts, this being attributed to the damping effects exerted by the heavy atoms of the inorganic anion (109).

In both the spectra of ammonium reineckate and the derivatives studied, complimentary, broad, intense bands occur throughout the 3500-2500 cm.$^{-1}$ and 700 cm.$^{-1}$ regions. This suggests that the anion is largely responsible for these modes.

Two marked absorptions are observed in the 2100 cm.$^{-1}$ and 1250 cm.$^{-1}$ regions (nitrile stretching vibrations) for all spectra. The 8-11 micron region appears to be specific for each derivative, and is of value for their qualitative identification.

10) Tetraphenylborates

Pellet concentrations of 1.1% were used for all derivatives, with the exception of cycrimine TPB which was determined at a concentration of 1.4%. Samples were shaken for fifteen seconds and the subjected to five minutes pressure.

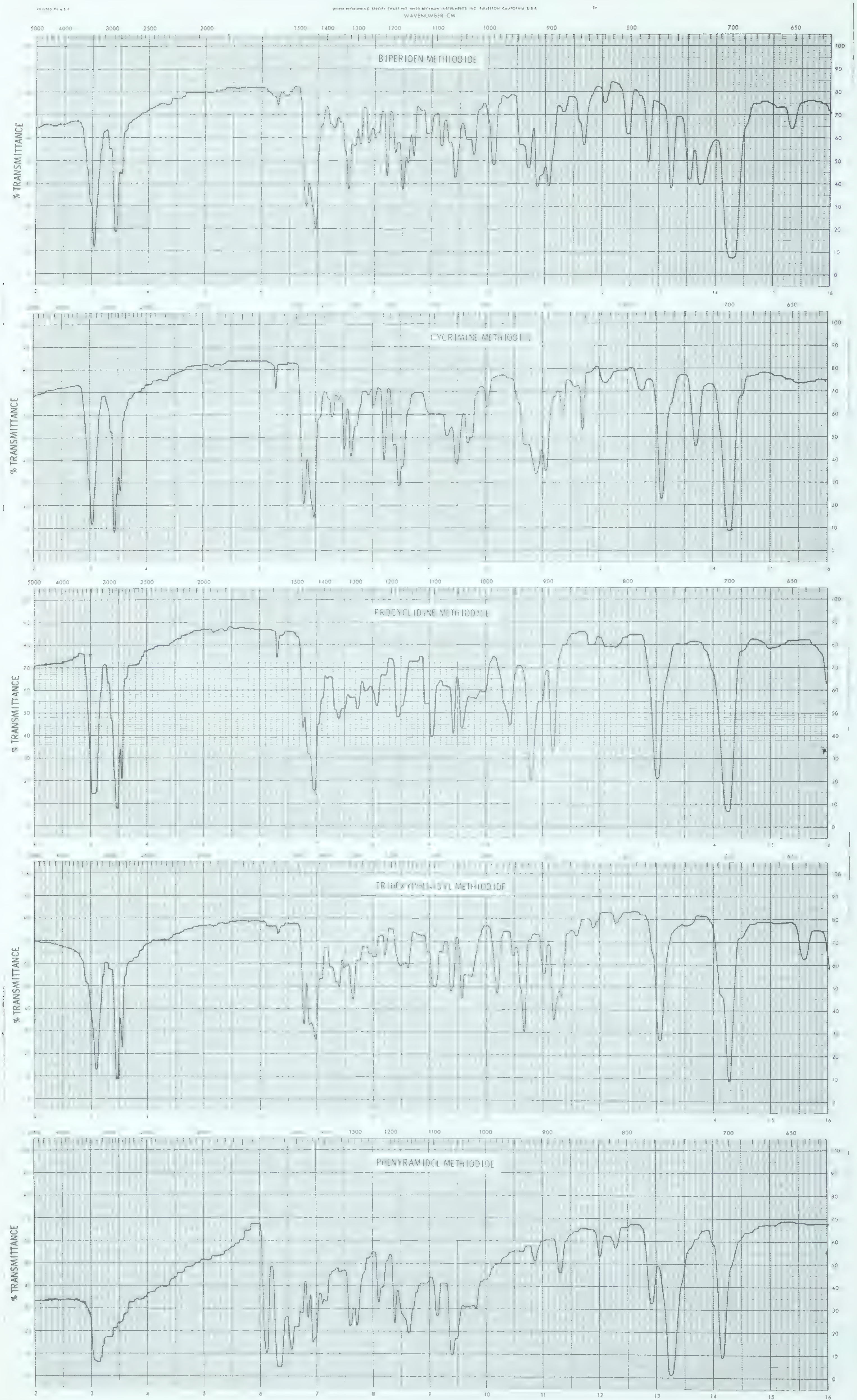


Figure 11. Infrared spectra of the methiodides.

The infrared spectra of the TPB compounds are shown in Figure 10. All spectra display a weak to medium band in the 3500-3400 cm.$^{-1}$ region due to OH stretching vibrations.

In the spectrum of sodium tetraphenyborate, the TPB anion exhibited characteristic bands attributed to the monosubstituted phenyl and boron-aryl groups (112), with two intense broad bands in the 750-700 cm.$^{-1}$ region (out-of-plane CH bending vibrations of the phenyl groups). These same bands occur in the infrared spectra of all of the TPB derivatives studied. Aromatic and aliphatic CH stretching vibrations are found throughout the 3000 cm.$^{-1}$ region.

The charateristic medium absorption ca. 2700-2250 cm.$^{-1}$ of the mineral acid salts of the muscle relaxants (Figure 13) is absent from the corresponding TPB spectra. Medium to strong absorption, appearing as either a single band or a doublet is observed in the 1600 cm.$^{-1}$ region. For the tetraphenylborate derivatives, the 1600-1500 cm.$^{-1}$ profile generally remains unaltered when compared with the spectra of the corresponding hydrochloride salts (exception being, procyclidine TPB). However, in the "fingerprint" region, which is more sensitive to total molecular structure, the absorptions are modified. Although there is a similarity in spectra because of the absorbance due to the TPB group, no two spectra are identical, thus permitting their application to qualitative differentiation.

11) Methiodides

Well resolved spectra could be obtained for the methiodides by using the following pellet concentrations: biperiden, cycrimine, procyclidine, phenyramidol 1.1%; trihexyphenidyl 1.4%. The shaking times were all of fifteen second duration and pellets

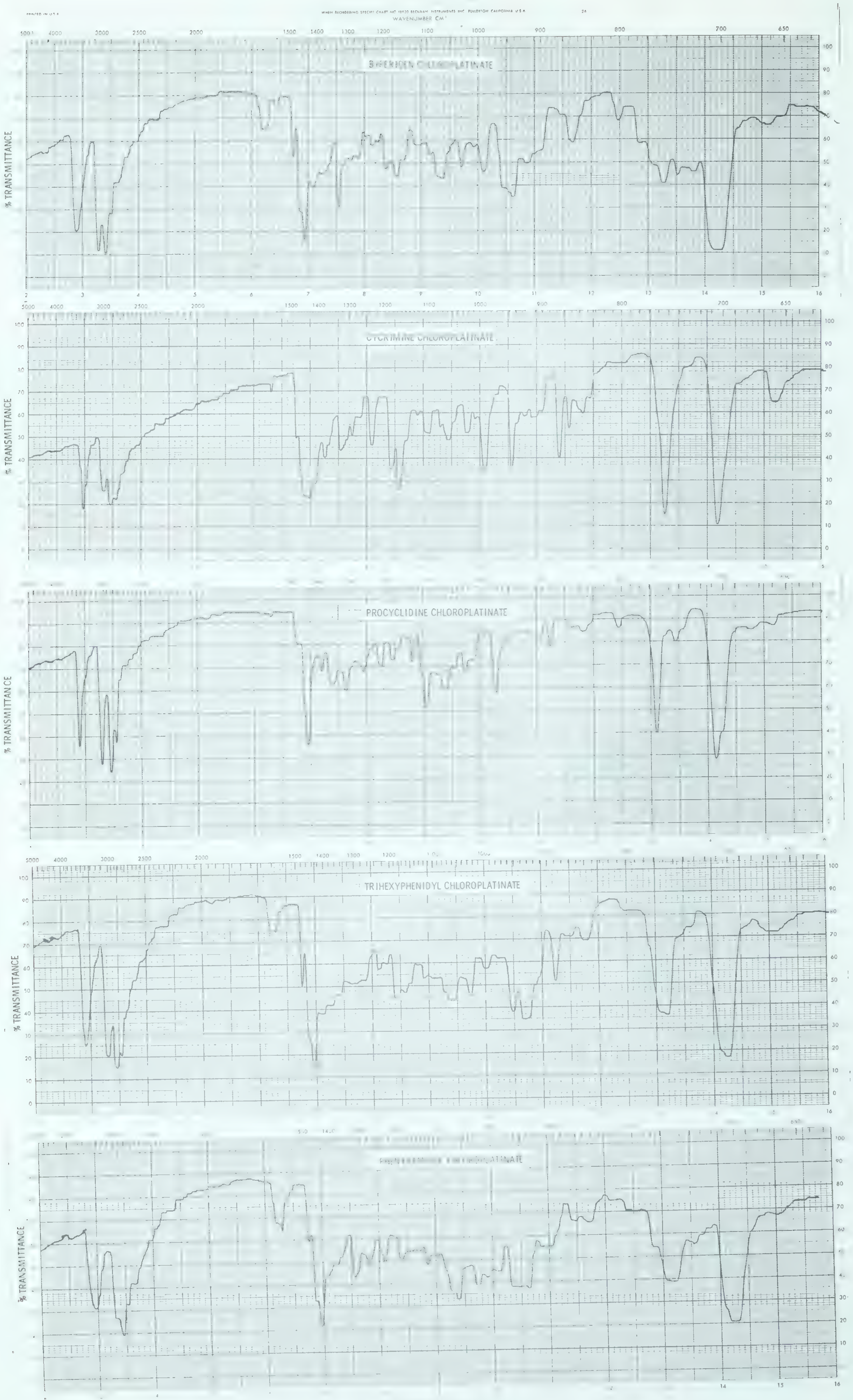


Figure 12. Infrared spectra of the chloroplatinates.

were pressed for five minutes.

These spectra (Figure 11) exhibit numerous common bands, in keeping with their structual similarity. Strong absorption bands at 3300 cm.$^{-1}$ due to OH stretching vibrations and medium to strong aromatic and aliphatic CH stretching throughout the 3050-2900 cm.$^{-1}$ regions are obvious with the exception of phenyramidol methiodide. Weak to medium C=C skeletal in-plane phenyl ring vibrations ca. 1600 cm.$^{-1}$ and 1500 cm.$^{-1}$ can be seen.

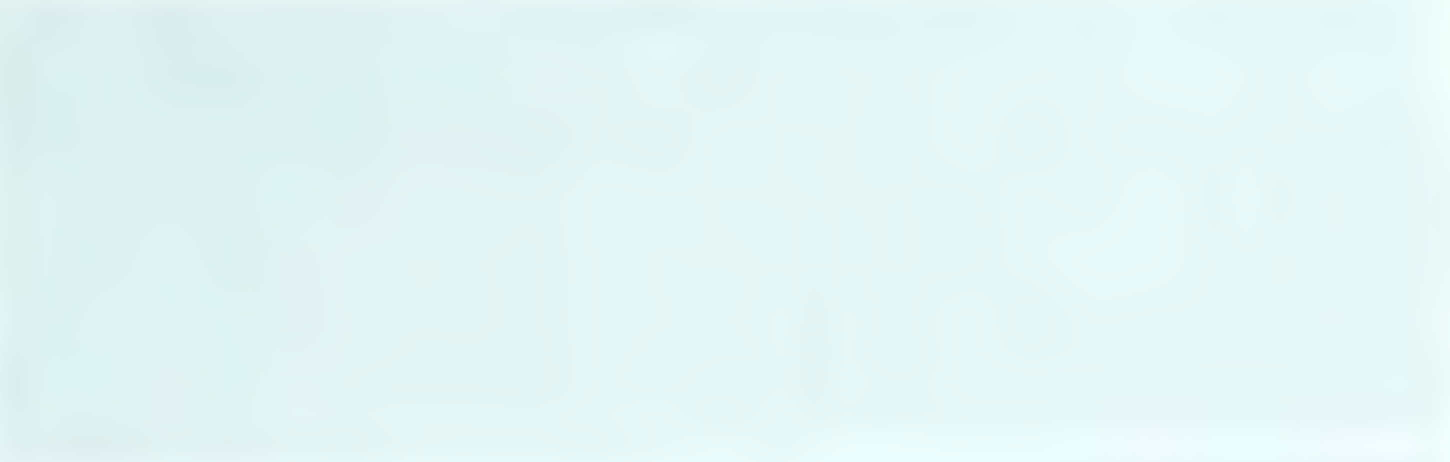
The 8-16 micron region, with its very charateristic CH out-of-plane bending bands in the lower frequencies and especially the 9-14 micron region, is most useful for differentiation of these compounds.

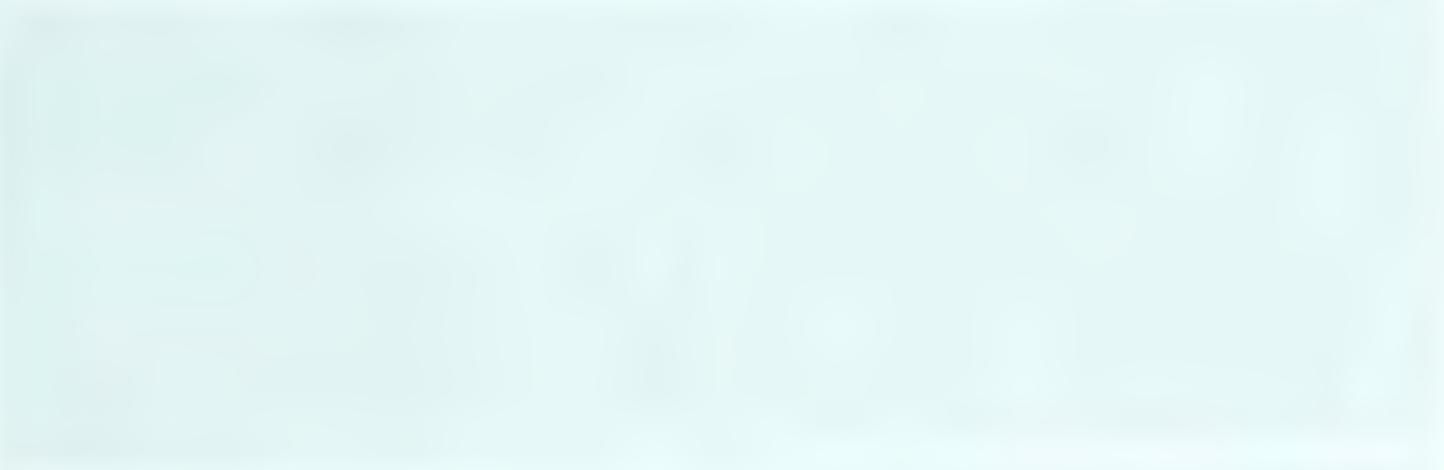
Unlike other amines, quaternary ammonium salts lack characteristic bands.

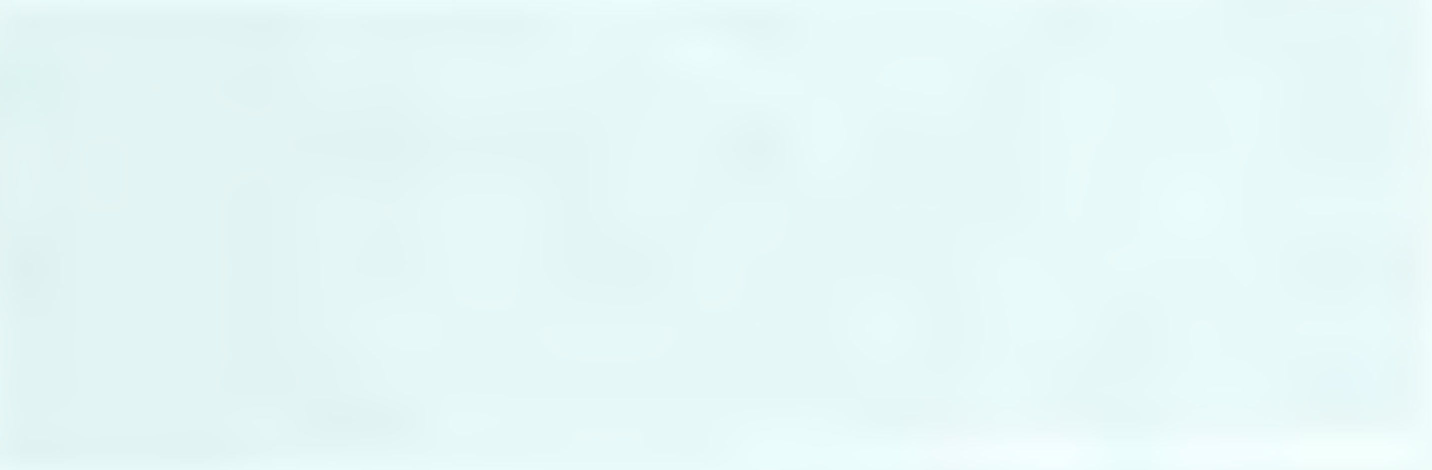
12) Chloroplatinates

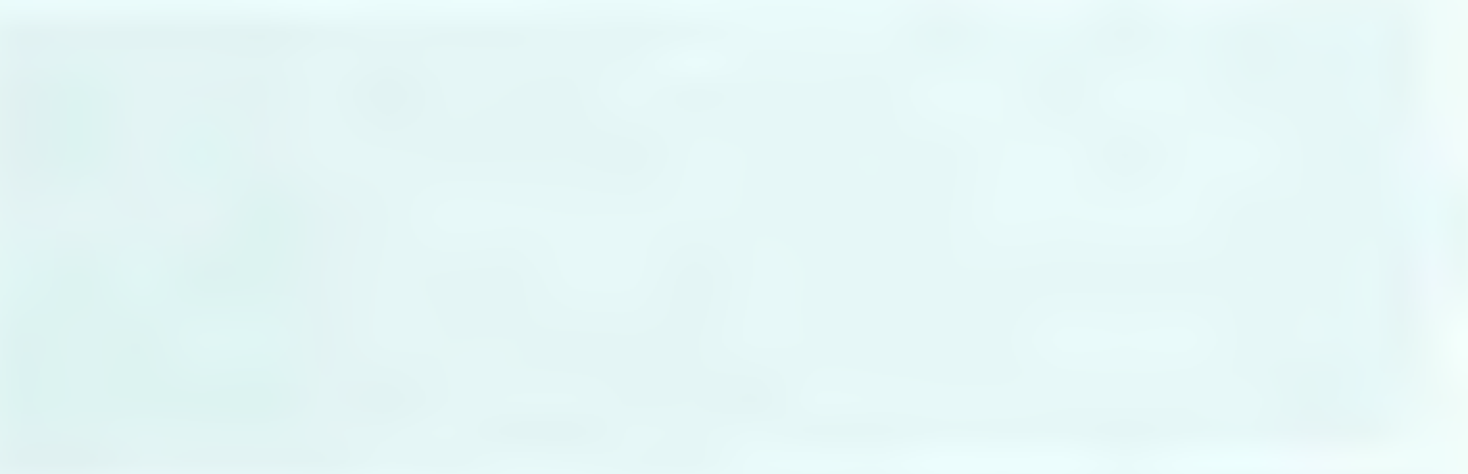
The chloroplatinate infrared spectra did not give good resolution,and in all instances lacked strong, well defined bands in the "fingerprint" region (Figure 12). Pellet concetrations ranging from 0.3 to 3.0% were tried for each derivative and unsatisfactory results were obtained in all instances. Higher concentrations did not result in the appearance of new or better resolution in the 10-16 micron region. The following pellet concentrations gave the best resolved spectra: biperiden 1.3%, cycrimine 1.5%, procyclidine 1.0%, phenyramidol 1.8%, trihexyphenidyl 1.9%. Samples were mixed briefly with a spatula before shaking for five seconds in the amalgamator and subsequent pressing for three minutes.

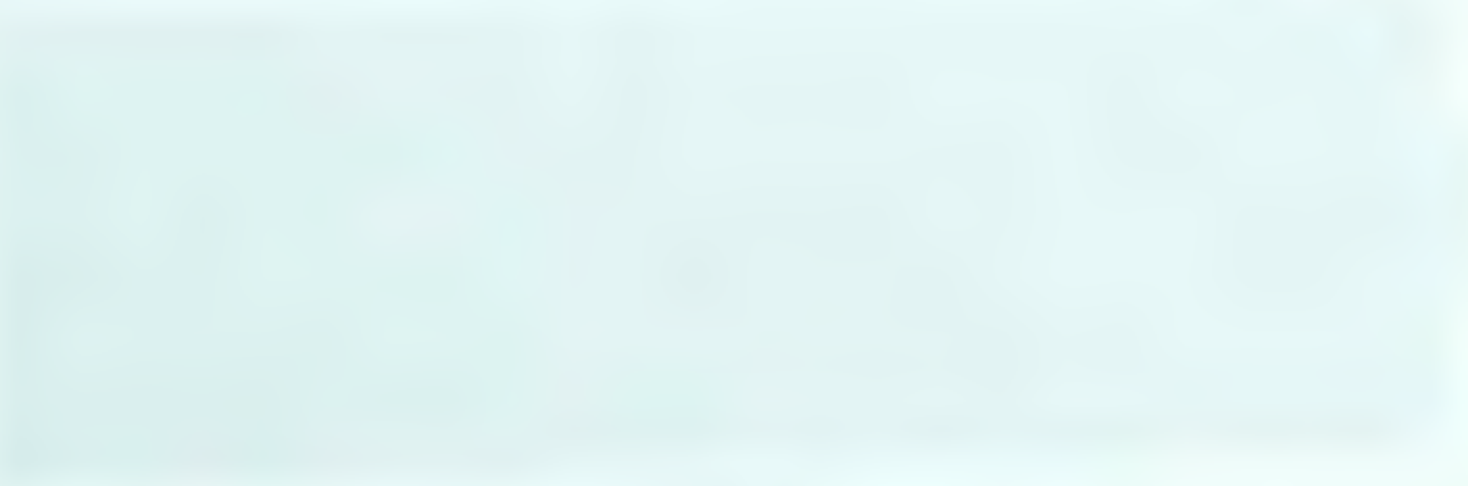
The spectrum of chloroplatinic acid revealed that this reagent











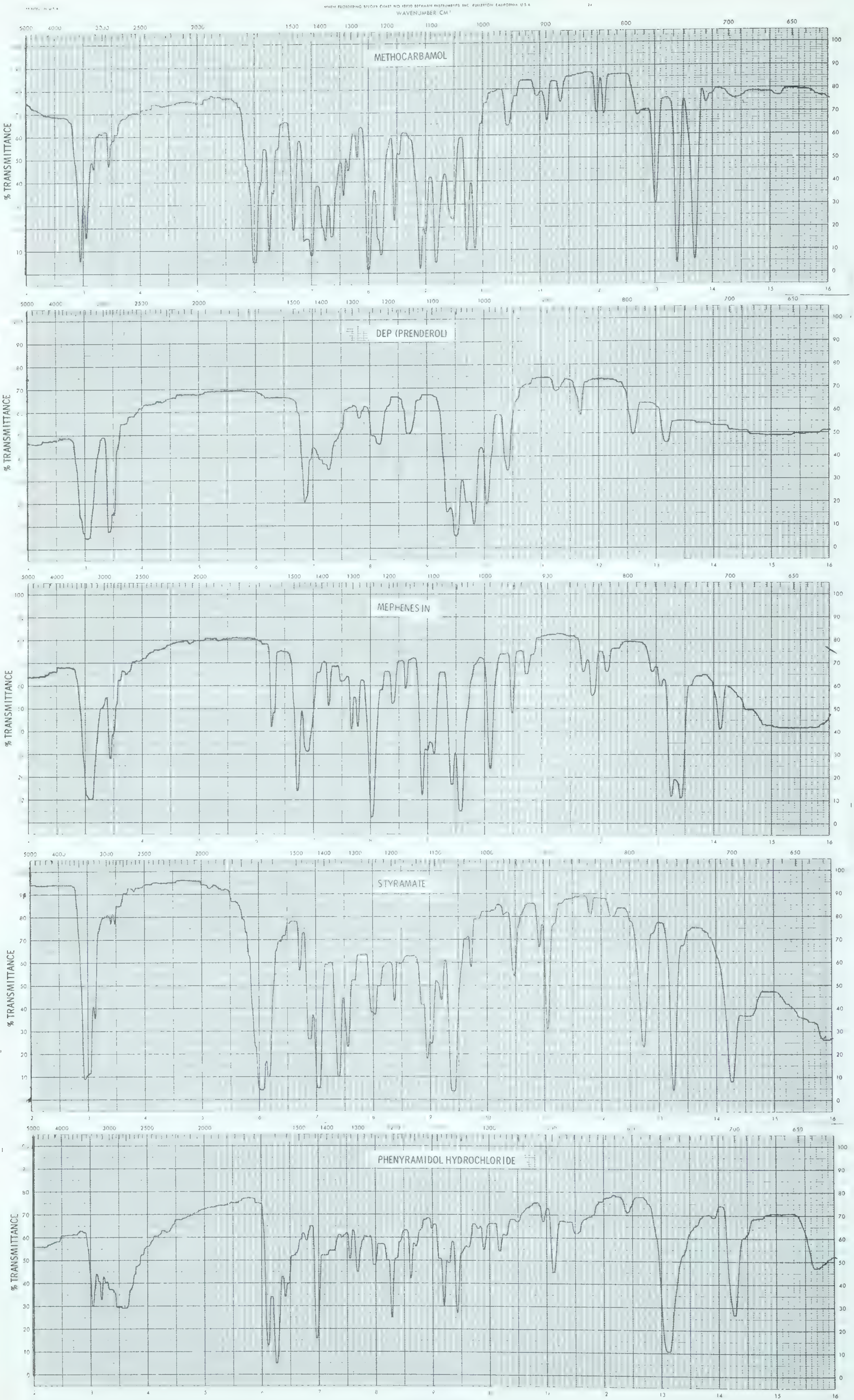
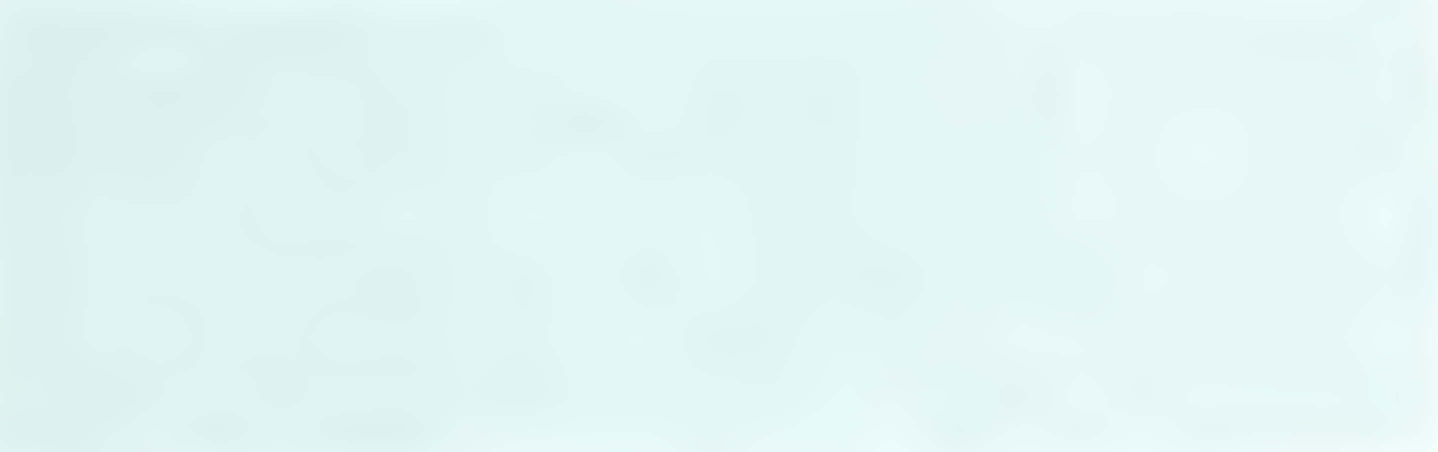


Figure 13. Infrared spectra of the parent compounds.



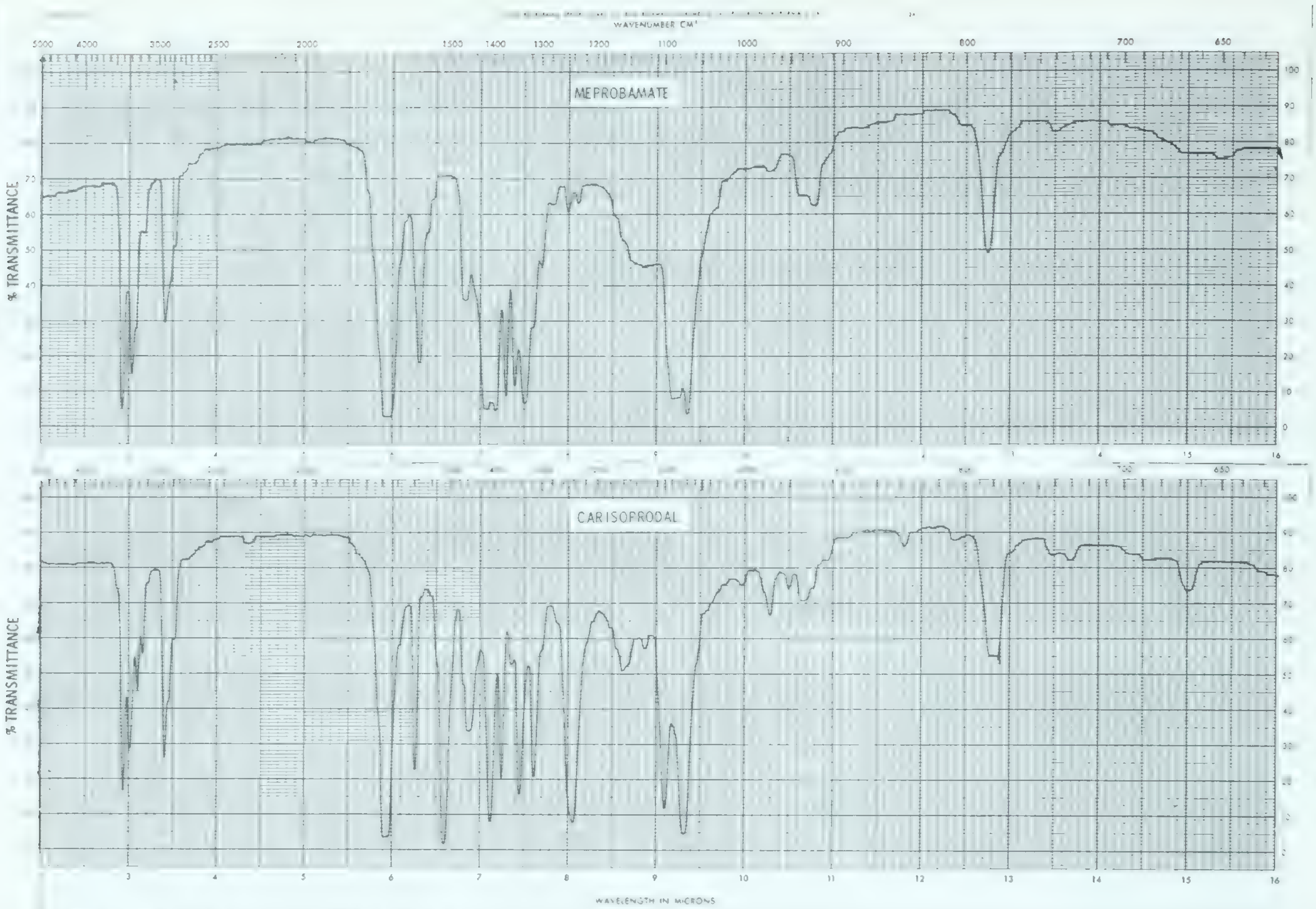


Figure 13. - Continued.

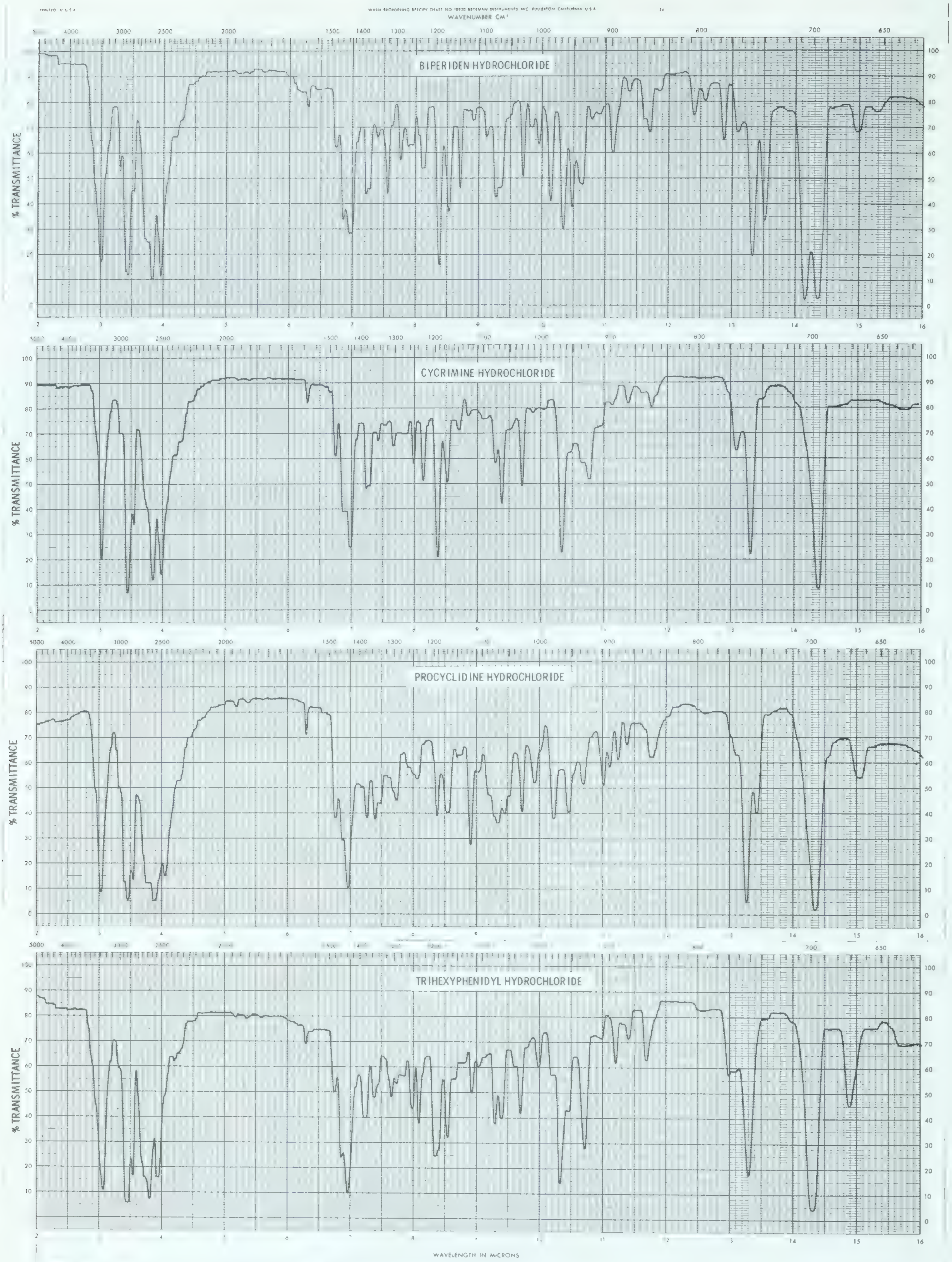


Figure 13. - Continued.

did not absorb in the infrared region (2-16 microns) with the exception of two "water bands" of medium and weak intensities at 3350 cm.$^{-1}$ and 1620 cm.$^{-1}$ respectively. Since the reagent contains water of crystallization these bands were not anomalous. It might therefore be reasonably concluded that the spectra of the chloroplatinate derivatives be identical with the spectra of the parent compound mineral salt. Some similarities were noticed but the poor resolution of the chloroplatinate spectra did not allow a proper comparison.

It is possible that the heavy chloroplatinate anion is responsible for this damping effect, also the fact that the chloroplatinate derivatives were obtained in an amorphous state and were not recrystallized could have resulted in poorly resolved spectra.

These spectra are of little utility to the qualitative identification and differentiation of these compounds but may give an indication.

13) Parent Compounds

The infrared spectra of the parent compounds are presented in Figure 13. Good resolution was obtained using the following pellet concentrations: methocarbamol 0.3%, DEP (prenderol) 0.33%, mephenesin 0.3%, styramate 0.3%, phenyramidol hydrochloride 0.75%, biperiden hydrochloride 0.75%, cycrimine hydrochloride 0.8%, procyclidine hydrochloride 1.4%, trihexyphenidyl hydrochloride 1.2%, meprobamate 0.3%, carisoprodal 0.3%. A shaking time of fifteen seconds was used for all samples with subsequent pressing periods of: procyclidine and cycrimine hydrochloride three minutes; mephenesin, styramate, methocarbamol, and biperiden, phenyramidol, trihexyphenidyl hydrochloride salts ten minutes; DEP seven minutes; carisoprodal and meprobamate fifteen minutes.

These spectra are presented as an additional paramater for identification, and examination can reveal structural features reflecting the presence of specific functional groups in the parent compounds.
Many of these characteristic bands are found in the spectra of the derivatives produced.

C. PHOTOMICROGRAPHY

Of the methods available for the identification of minute quantities of basic organic nitrogenous materials, the production of microcrystalline derivatives of characteristic form and habit is generally acknowledged to be one of the most sensitive and reliable. In addition, the use of chemical microscopy as an adjunct to organic qualitative analysis adds greatly to the ease of identification (137). A broad variety of nitrogenous pharmaceutical bases have been characterized by this means (138, 139,140,141,94), and the technique has proven of value in the identification of the amine containing muscle relaxants included in this thesis.

A large number of reagents were employed in an attempt to obtain complete and comparative sets of photomicrographs. However, success was limited to only a few of these, and the pattern of results was irregular. All photomicrographs were taken at 50X magnification.

It must be understood that the crystalline habits of these compounds are not to be used as a sole criterion for identification, but rather as an adjunct to other physico-chemical methods previously discussed (melting points and the infrared spectra of selected derivatives).

Only the characteristic photomicrographs have been reproduced and nonspecific amorphous precipitates, oils or crystals, which often formed, have been deleted.

The microcrystal derivatives obtained will be briefly discussed under the names of the amine salts produced. Parent compound may hereafter be abbreviated to "P.C." in the tables.

1) Picrates and Styphnates

In only one instance were efforts to prepare characteristic microcrystals with either picric or styphnic acid successful, as is apparent from Table XVII. Phenyramidol hydrochloride yielded well defined microcrystals with each agent and photomicrographs are given in Figure 14. Other parent compounds (biperiden, cycrimine, procyclidine, and trihexyphenidyl), immediately precipitated as oils or oily films which would not crystallize. Varying concentrations of aqueous and alcoholic parent compound and reagent solutions were used with no success.

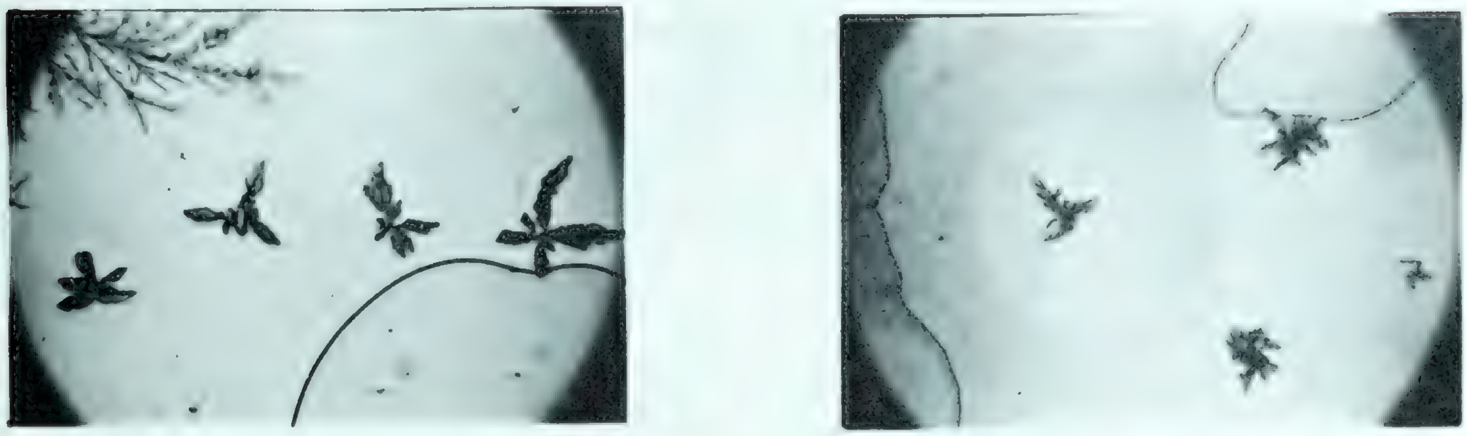
Table XVII. Microchemical Tests with Picric Acid and Styphnic Acid

Derivative	Reagent Conc. (%) (P.C. + Reagent)	Description and Characteristics of Microcrystals
Phenyramidol Picrate	0.5 + 0.5	Yellow feathery blades which formed within 10 minutes
Phenyramidol Styphnate	0.5 + 0.64	Yellow jagged irregular blades which formed within 15 minutes

2) Chloroplatinates

Chloroplatinic acid, in various concentrations, did not prove to be a satisfactory reagent for the identification of all amine muscle relaxants studied. Well defined, distinctive crystals were obtained for only two of the five compounds investigated (cycrimine, procyclidine). The others formed a light amorphous precipitate which was not characteristic or acceptable for the identification of these drugs. Clarke (94) has prepared the chloroplatinate of cycrimine and his description complimented our findings.

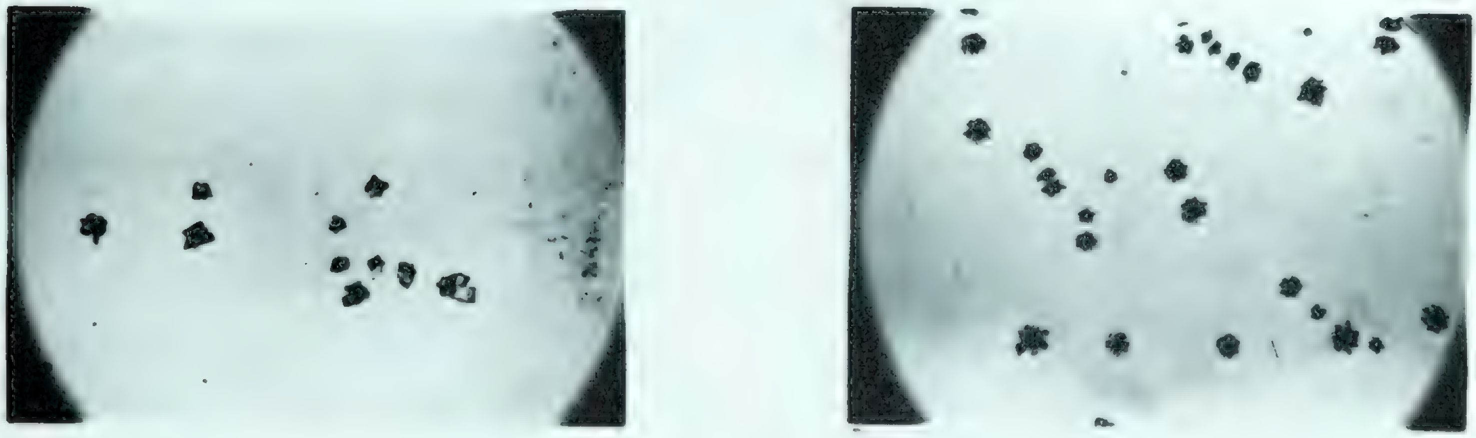
The resulting derivatives are discussed in Table XVIII and the corresponding photomicrographs are found in Figure 15.



Phenyramidol Picrate
50X

Phenyramidol Styphnate
50X

Figure 14. Photomicrogaphs of the picrates and styphnates.



Cycrimine
50X

Procyclidine
50X

Figure 15. Photomicrographs of the chloroplatinates.

Table XVIII. Microchemical Tests with Chloroplatinic Acid

Parent Compound	Reagent Conc. (%) (P.C. + Reagent)	Description and Characteristics of Microcrystals
Cycrimine	1.0 + 1.0	Pale yellowish orange bunches of plates and prisms which formed within 5 minutes
Procyclidine	0.5 + 1.0	Pale yellow rosettes of irregular blades which formed quickly within 3 minutes

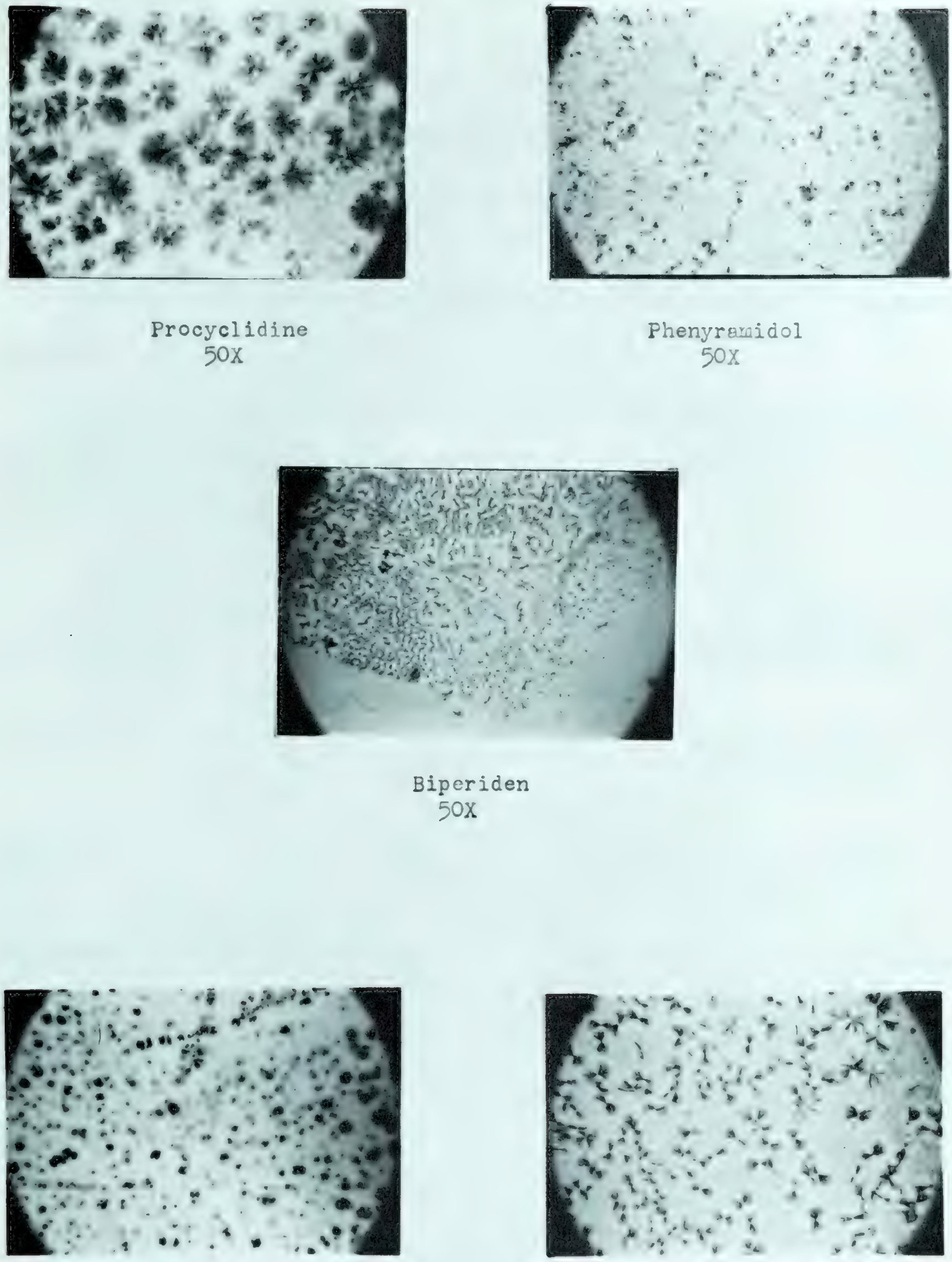
3) Reineckates

Ammonium reineckate, in various concentrations, proved to be an excellent reagent for the identification of the amine muscle relaxants since characteristic and distinctive crystals were obtained for all five of the parent compounds investigated.

The reineckates prepared are discussed in Table XIX and the corresponding photomicrographs are presented in Figure 16.

Table XIX. Microchemical Tests with Ammonium Reineckate

Parent Compound	Reagent Conc. (%) (P.C. + Reagent)	Description and Characteristics of Microcrystals
Procyclidine	0.5 + 0.5	Mauve rosettes of radiating fine needles which formed within 5 minutes
Phenyramidol	0.5 + 0.5	Purplish crystalline masses having no characteristic gross morphology and remaining unresolved after 20 minutes
Biperiden	0.1 + 0.1	Small pale violet "bow-tie" conformations of fine needles which formed in about 30 minutes
Cycrimine	0.5 + 0.5	Very small purple rosettes of short fine needles forming within 5 minutes
Trihexyphenidyl	0.5 + 0.5	Larger mauve "bow-tie" conformations of fine feathery needles which formed in about 10 minutes



Procyclidine
50X

Phenyramidol
50X

Biperiden
50X

Cycrimine
50X

Trihexyphenidyl
50X

Figure 16. Photomicrographs of the reineckates.

4) Picrolonates

Picrolonic acid, proved to be a satisfactory reagent for the characterization of four of the five compounds studied. Clarke (94) has prepared the picrolonate of procyclidine and favorable agreement was noted with this investigation.

Results of the present study are found in Table XX and photomicrographs are presented in Figure 17.

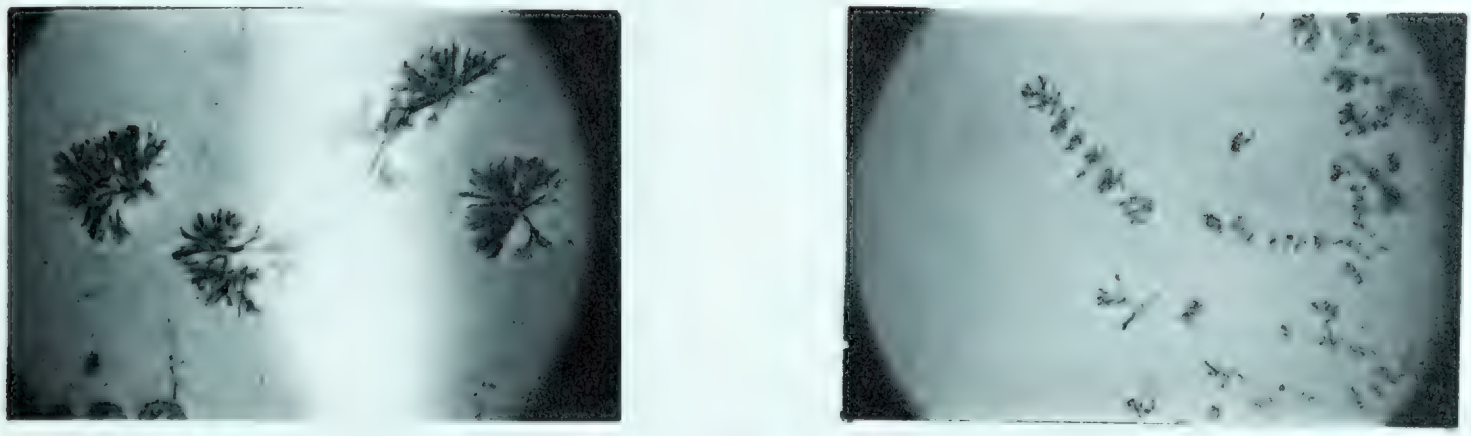
The picrolonate of phenyramidol could not be obtained in crystalline form under all the varied aqueous and alcoholic conditions used. The material invariably precipitated as a golden yellow oil.

Table XX. Microchemical Tests with Picrolonic Acid

Parent Compounds	Reagent Conc. (%) (P.C. + Reagent)	Description and Characteristics of Microcrystals
Procyclidine	0.5 + 1.0 (alc.)	Pale orange bushy clusters of dendrites and branching needles forming within 5 minutes
Biperiden	0.1 + 0.25 (alc.)	Yellowish orange irregular branching needles forming within 5 minutes
Cycrimine	0.5 + 1.0 (alc.)	Light yellow irregular blades forming within 5 minutes
Trihexyphenidyl	0.5 + 1.0 (alc.)	Fine pale orange overlaping needles and rods forming within 5 minutes
Phenyramidol	...	Golden yellow oil which remained unchanged after 20 minutes

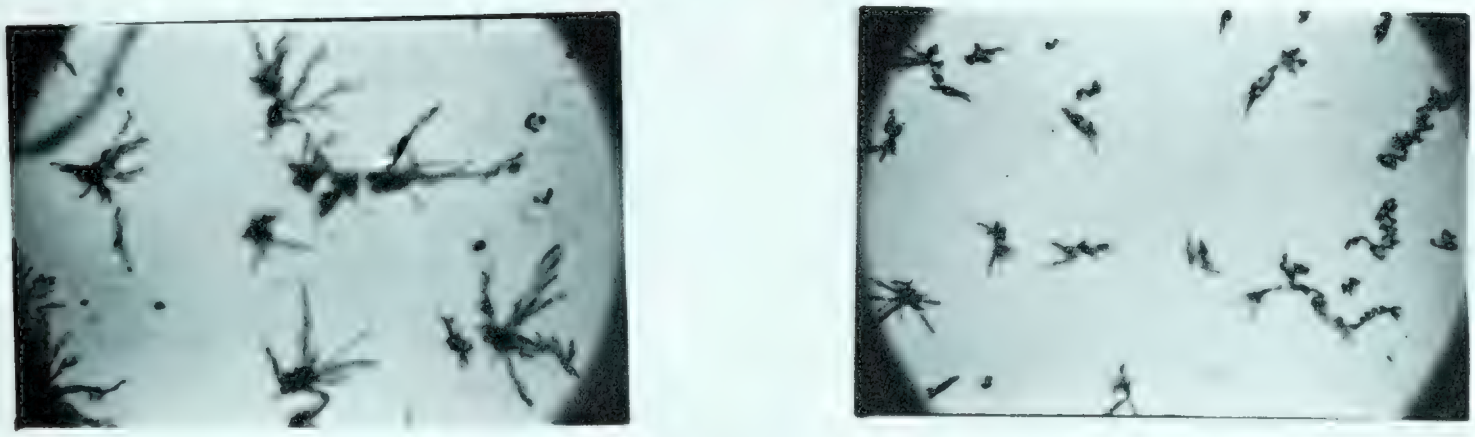
5) Hydroiodides

The method of Hucknell and Turfitt (142) was used for the preparation of these microcrystals. As is evident from Table XXI satisfactory crystalline results were obtained for four of the five compounds studied. These microcrystals were easily and rapidly prepared and gave, characteristic photomicrographs (Figure 18).



Procyclidine
50X

Biperiden
50X



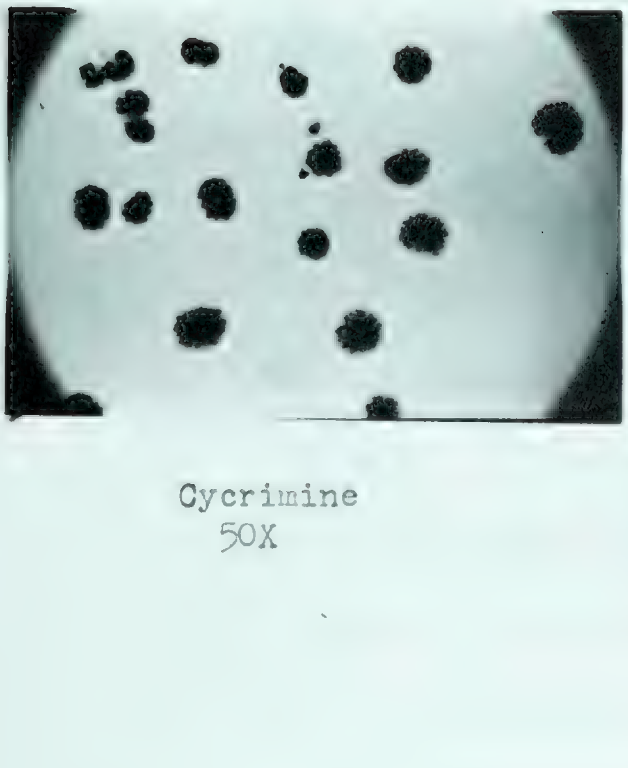
Cycrimine
50X

Trihexyphenidyl
50X

Figure 17. Photomicrographs of the picrolonates.

Procyclidine
50X

Biperiden
50X



Cycrimine
50X

Trihexyphenidyl
50X

Figure 18. Photomicrographs of the hydroiodides.

Phenyramidol was the only anomaly and this yielded a fine amorphous precipitate which would not crystallize on standing.

Table XXI. Microchemical Tests with Potassium Iodide

Parent Compound	P.C. Conc. (%)	Description and Characteristics of Microcrystals
Procyclidine	1.0%	Large transparent blades radiating from a central nucleus and forming in about 5 minutes
Biperiden	0.1%	Small dark rosettes of thread like needles radiating from a central nucleus and forming in about 15 minutes
Cycrimine	0.5%	Large dark "powder-puff" rosettes of fine needles forming in about 1 minute
Trihexyphenidyl	1.0%	Large white rosettes of fine thread like needles radiating from a central nucleus
Phenyramidol	...	White opaque film of amorphous precipitate which formed immediately and would not crystallize

6) Unsuccessful Microchemical Tests

During this study several other alkaloidal of basic nitrogenous reagents were investigated. These included gold chloride, potassium permanganate, sodium tetraphenylborate, potassium dichromate, and Dragendorff's reagent. In each instance, nonspecific amorphous precipitates, oils or crystals were formed which were of no value for the identification and differentiation of the five amine muscle relaxants.

In summary, the characteristic and selective microcrystals formed with the various reagents are indicated by a plus sign in the following table (Table XXII).

In conclusion, it is apparent that the amine muscle relaxants can be differentiated by the use of microcrystallography but an identification based solely on the reaction of any one of the reagents is ill advised.

Since different basic nitrogenous compounds will on occasion form similar crystal or precipitate patterns, it is recommended that for qualitative identification purposes, two or more of these reagents should be used.

Table XXII. Amine Muscle Relaxants Characterized by Microcrystallography

Parent Compound	Picric Acid	Styphnic Acid	Ammonium Reineckate	Platinic Chloride	Picrolonic Acid	Potassium Iodide
Phenyramidol	+	+	+			
Cycrimine			+	+	+	+
Biperiden			+		+	+
Procyclidine			+	+	+	+
Trihexyphenidyl			+		+	+

CONCLUSIONS

1. A series of specific physical criteria, by which eleven of the newer muscle relaxants currently in use can be positively identified and differentiated, has been presented.

2. Fifty five derivatives of these drugs have been prepared in a systematic manner, of which forty eight have not been reported to date in the literature.

3. The pertinent physical data of these derivatives and the parent compounds have been presented in Tables I - XIII.

4. The infrared spectra of these derivatives and their parent compounds have been obtained as a further parameter for their qualitative differentiation and are outlined in Figures 1 - 13.

5. A series of photomicrographs for five muscle relaxants containing an amine moiety have been included in Figures 14 - 18, along with descriptive data concerning their formation and gross morphology in Tables XVII - XXI.

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